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A COMPARATIVE STUDY OF CERTAIN TISSUES OF GIANT-HILL AND HEALTHY POTATO PLANTS[1]

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REVIEW OF LITERATURE

Folsom (9) in 1926, reporting on viroses of the potato, classified them in 5 groups as follows: leaf roll, mosaic, aucuba mosaic, streak, and spindle tuber. The last-named group includes the malady known as giant hill. Though giant hill is thus classed as a virosis, Folsom (9) says it "has not yet been definitely proved to be transmissible from plant to plant" and that the diseases of this spindle-tuber group have not been tested for the presence of a pathogen with a known type of structure.

Mention of giant hill as a potato disease was first recorded in 1924 (24) as an "abnormal type of potato plant which Dr. E. L. Nixon discovered several years ago and to which he has given the apt name of Giant-Hill." The same year Folsom (8) also refers to the isolation of "another symptom aggregate . . . that breaks up tuber-units and is perpetuated in the tubers, and that seems to answer the typical description and some of the hill selection examples of Nixon's Giant-hill degeneration disease." In 1924 Gilbert (12), too, records a condition that has been "referred to as 'Giant Hill.'" Writing in 1925, Gilbert (13) called giant hill a "new phase of spindle tuber." In 1924, Bailey (4), in Vermont, and McCubbin, Hartman, and Lauer (19) in 1925 included giant hill among the serious diseases of the potato. Since then it has become recognized as a disease that, though not of equal importance in all potato-growing regions, is sufficiently serious to receive attention.

Since the gross appearance presented by giant-hill potato plants and certain tuber characteristics have been their chief distinguishing features, it may be well here to list some of them.

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The following composite description of giant hill, comprising the essential characters presented by various authors (10, 12, 13, 18, 20), will suffice to acquaint the reader with the disease. The root system shows an abnormal fibrous development, with numerous and thicker roots as well, and the stolons are notably longer than in normal plants. The tubers, generally formed later, are of undesirable shapes for seed and market purposes, with constrictions, deep eyes, and heavy shoulders back of the eyes. One author (12) relates giant hill to spindle tuber on the basis of the similarity of their tuber shape. Usually there are one or two large, poorly shaped tubers and a number of very small ones.

Conflicting reports as to the effect on yields can perhaps be harmonized[3] by a consideration of environmental conditions. In Maine, Folsom (9) and Folsom *et al.* (11) report that the yield may be slightly less or as much as 20 per cent greater than normal. In Pennsylvania (19) giant hill is becoming an important limiting factor in commercial and seed crop production. Goss (14) and Goss and Peltier (15), as well as Nixon, believe that environment affects the symptoms, spread, and effect on yield of potato-degeneration diseases. Conditions adverse to good potato growth, such as drought, result in giant hill, in the building of vine and not tuber.[3] When conditions are favorable for potato growth, as they usually are in Michigan and Maine the giant hill characteristics are less marked.

Figures of total weights from 22 hills each in 1921 indicate the effect of giant hill on yield in Pennsylvania. Each hill had been planted from a separate potato, the seed pieces being equivalent in weight and comparably planted. Twenty-two hills of healthy plants from Lehigh County, produced 27.44 pounds of potatoes. Twenty-two hills of giant-hill plants from Cambria County produced 13.18 pounds, and the same number of giant hills from New York State, 14.63 pounds.

The aboveground portion of the giant hill plant generally is coarse, with excessive branching (20, 9), and vines taller than normal. (Fig. 1). During the early part of the growing season giant hill plants sometimes appear lighter green than healthy plants, but with the advancing season they become relatively darker and remain green and upright after other vines have died. The tendency of the vines to remain green and upright after other vines have died and to be resistant to the early frosts that kill most potato plants makes it possible at the end of the season to recognize giant hill and to rogue seed fields for the disease.

Giant-hill plants usually consist of but a single and very stiff stalk (31), of erect habit and an excessive amount of lateral growth proceeding from numerous axillary shoots (20). Nixon[3] refers to the "constriction of the

[3] Personal communication from E. L. Nixon, Professor of Plant Pathology, The Pennsylvania State College.

tops" of giant-hill plants, by which he means the more erect and compact habit, especially at the top of the plant as compared with the spreading nature of the healthy plants. The leaves are coarse with an irregular surface and pronounced purple tinge along the edges. In Rurals, the gloss and purple coloring of giant-hill vines is particularly noticeable.

According to Gilbert (12) the leaves are not rugose nor marginally inrolled, but Folsom (9) says rolling may be "slight or absent." Though slight chlorosis may be characterisitc of the leaves (9) an account by Lauer (18) describes the foliage as dark green. A later and more prolonged blossoming period generally is observed in giant hill plants. In general, "all the characteristic symptoms of giant hill are those of excessive vegetative growth" (19).

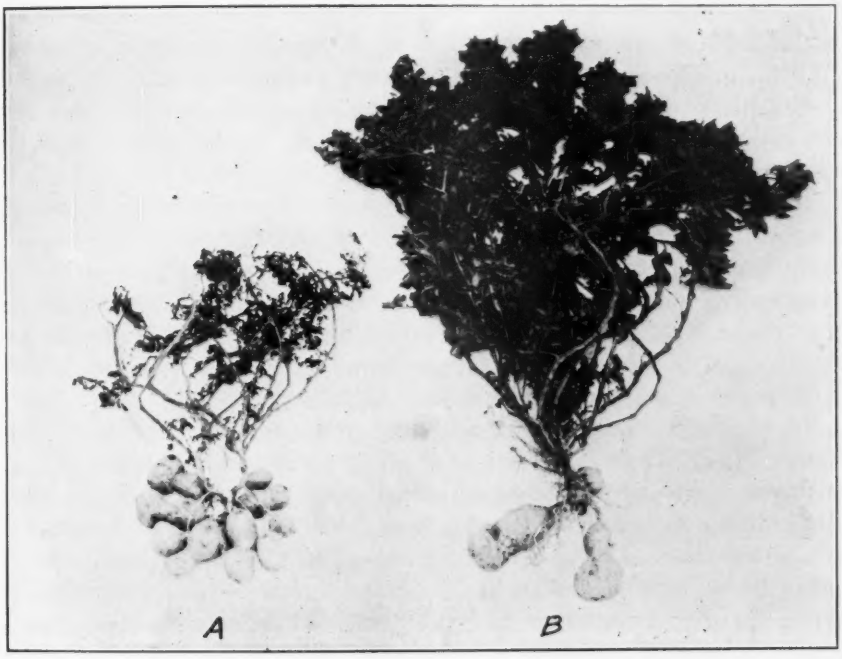

FIG. 1. *A*. Healthy plant with tubers. *B*. Giant-hill plant with tubers.

Artschwager (1), preliminary to the study of the pathological anatomy of plants suffering from various diseases, made a very comprehensive investigation of the anatomy of the potato plant. The bicollateral bundle, one in which there is phloem both outside and inside of the xylem, is one of the outstanding characteristics of the stem. Artschwager points out that the preponderance of sieve tubes in the secondary phloem makes the importance of the phloem in translocation appear self-evident. With the phloem occu-

pying such a strategic position in the healthy functioning of the potato plant, any disturbance in this tissue assumes a critical aspect.

Various investigations have shown histological relationships with diseased conditions. Quanjer (25) was the first to describe phloem necrosis in leaf-roll plants, a condition confirmed later by Artschwager (2). Disorganization in phloem, xylem, parenchyma, necrosis in cortex, collenchyma, and epidermis were reported also by Quanjer (22) in "streak." Other investigators (7) report a failure to find anatomical differences, but point out that potato phloem appears to be particularly sensitive in showing disorganization phenomena. Artschwager (3) emphasizes that it is the universality in distribution of changes in the phloem coupled with absence of necrosis in other tissues that gives such changes diagnostic value. Though von Brehmer (5) differentiated a "necrobiosis" in all potato plants with well developed phloem, he also mentions Quanjer's phloem necrosis. Phloem necrosis is regarded by Thung (32) as a symptom of leaf roll in a far advanced stage, with transport having been disturbed before the destruction became visible. Of a number of tuber-producing plants that put a heavy demand on food movement, curiously the phloem of the potato alone is subject to degeneration (30).

Some investigators have regarded the appearance of necrosis in phloem as an age phenomenon; but Rochlin (27) reports that, in mosaic-diseased potato plants, necrosis of the phloem occurs much earlier than in healthy plants, where it is found normally at maturity. In addition to various other changes, Rochlin reported necrosis of pith and cortical cells and shortening of the palisade and spongy parenchyma cells with almost complete absence of intercellular spaces.

These observations on the palisade cells confirm previous reports. Iwanowski (17), as early as 1903, had agreed with Woods (34) that palisade cells were shortened in tobacco leaves affected with mosaic and the intercellular space reduced. Melchers (21) failed to confirm Woods' observations on the absence of palisade parenchyma in the light colored areas of tomato leaves showing mosaic and its modification in moderately diseased plants; he did, however, report modifications of the palisade tissue in potato mosaic similar to those found by Woods in tomato and tobacco.

A very recent investigation by Verplancke (33) on spindle tuber, a degeneration disease classified by Folsom (9) with giant hill as one group of potato viroses, showed that spindle tuber had the effect of increasing the ratio of length to width of the cells of all the tissues of the tuber, or an increase in length and decrease in width over the cells of the healthy potato. He believes that the elongation of the tuber in spindle tuber is a specific result of the action of the causative virus.

Reports of previous investigations on the histological and anatomical

effects of various degeneration diseases of plants, though not altogether in agreement, seem to have shown definite changes principally in the palisade tissues of the leaves and in the phloem of the stem. These changes have involved more or less disorganization of the tissues concerned.

METHODS

Since the present study was undertaken to find out whether the giant-hill plants had any anatomical or histological characteristics that might be correlated with this fairly distinctive picture presented in the field, sections were secured of leaves, petioles, and stems of both giant-hill and healthy potatoes. In 1927, E. L. Nixon brought in samples from various potato fields in Pennsylvania. A small number of plants from his plot was studied in 1928. For the investigation of material collected in 1930, samples were taken from plants in one of the experimental plots on the college campus. These plants were grown from tubers from giant-hill and healthy plants secured by E. L. Nixon and L. T. Denniston from various fields in the State. The seed pieces were planted in alternate rows of giant-hill and healthy tubers. The plants contributing samples to this study were checked by Nixon for their continuing giant-hill or healthy character at 3 successive times during the growing season and again at harvest. The samples from giant-hill and healthy plants were secured at the same time, thus insuring their equivalent age. For the 1932 study, samples were secured both from plants on one of the experimental plots on the college campus and from a plot in Potter County. Precautions similar to those in 1930 were observed as to the identity of the plants.

The material studied in 1927 consisted of stems, petioles, and leaves. The plant portions were fixed in an acetic acid-formalin-alcohol solution, and imbedded in paraffin, following the usual alcohol-xylol series. The 1930 material was mainly secured in July and was similarly fixed, but was imbedded in paraffin following treatment with an ethyl alcohol-butyl alcohol series (Zirkle 35). The harder pieces of stem from the lower parts of the plants were treated with hydrofluoric acid after fixing and some of them were imbedded in celloidin instead of paraffin. The 1932 material was secured in early September and was treated like that obtained in 1930.

Leaf sections were cut 10–12μ, petiole and stem sections, due to their hardness, from 15–22μ thick. The safranin-light green stain was used for all sections cut from material imbedded in paraffin and iron alum-haemotxylin was used to stain sections cut from material imbedded in celloidin. Somewhat more than 3800 slides were examined in the study.

In 1927 micrometric measurements were made of (1) entire cross sections of leaves, thickness of epidermis, collenchyma, outer phloem, inner phloem and xylem in the widest region of petioles and stems; (2) length

and width of palisade cells from leaf cross sections, of diameter of palisade cells from leaf sections cut parallel to the surface; and (3) diameter in cross section of parenchyma cells of stems.

In 1930, the measurements were confined to entire cross sections of leaves, of outer and inner phloem and xylem where it was widest in cross sections of petioles and stems, of pith and entire stem diameters, and of length, width, and diameter of palisade mesophyll cells in both giant-hill and healthy plants.

In 1932, measurements were made of entire cross sections of leaves, of length and width of palisade mesophyll cells, and of outer and inner phloem and xylem of stems.

Because of the time involved in securing sections, the study was restricted to a few sections of a comparatively small number of plants. Though hundreds of measurements were made of the material secured in 1927, these were regarded merely as indications, which were used as a foundation for the study of the 1930 material, and are not here presented. The statistical study, the results of which are given here, includes the material secured in 1930 and in 1932. This restriction was justified by the carefully repeated checking as to characteristics of the diseased or healthy condition of the plants studied in 1930 and 1932 compared with the single judgment used on the samples secured in 1927.

Of the leaves, as many as 50 measurements were made of a single leaf or 50 measurements of palisade cells from a single leaf. In the later material only 10 measurements were made. In cross sections of petioles and stems 10 measurements per plant were made. Tables were arranged from these measurements, and the means and other necessary calculations were based upon them. Odds were calculated from the table of Pearl and Miner (23). In 1930, 17 healthy and 31 giant-hill plants were the basis of the statistical study and in 1932, 14 healthy and 16 giant-hill plants were studied.

RESULTS

The tables[4] made of some of the measurements secured show that the material is highly variable, and calculations of the coefficients of variation confirm this indication. The percentages of variation are about the same for similar tissues in both the diseased and healthy plants. Determinations of probable errors and odds show, however, that, notwithstanding the variability, there are a few fairly consistent differences in some of the tissues of the giant-hill and healthy plants sampled. For the sake of greater accuracy, calculations were made in terms of micrometric measurements. The means have been translated to millimeters.

[4] Available for consultation on application to the Department of Botany, The Pennsylvania State College.

Leaf thickness was regarded in 1927 as indicating promising differences.

Measurements of both the 1930 and 1932 material showed that the leaves of the healthy plants were thicker than those of the diseased. A more careful analysis of the 1930 material (31 plants) showed that the mean

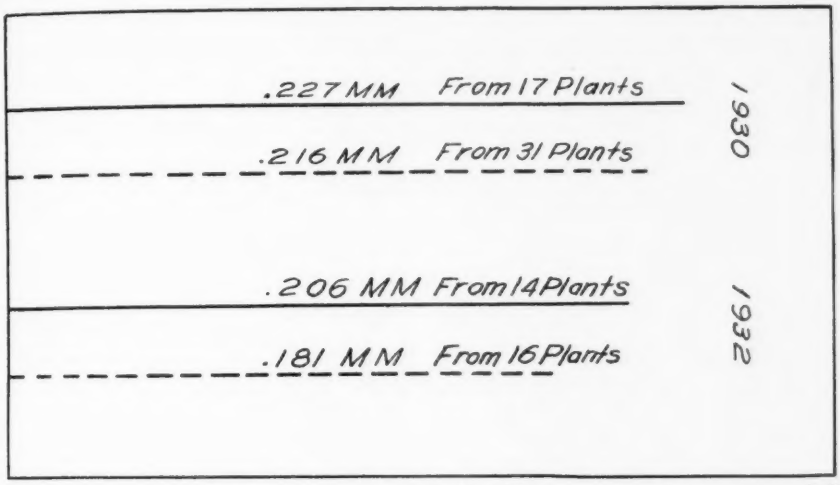

FIG. 2. Comparative measurements of leaf thickness expressed in means. Solid line for healthy, broken line for giant-hill plants.

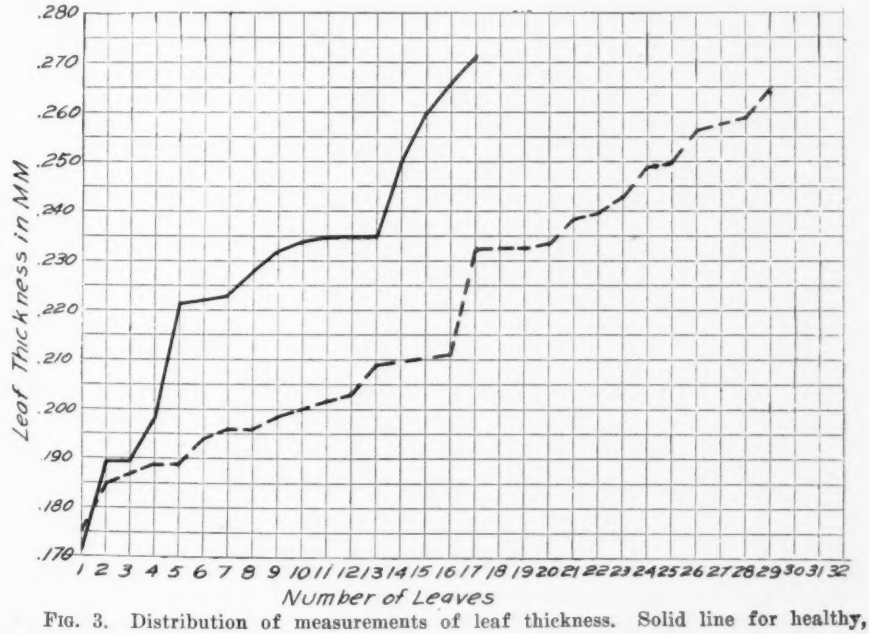

FIG. 3. Distribution of measurements of leaf thickness. Solid line for healthy, broken line for giant-hill plants.

of the thickness of the giant-hill leaves is 0.21665 mm. and that of the healthy (17 plants) is 0.22785 mm. In terms of micrometer spaces the mean thickness of the giant hill leaves is 12.38 ± 0.063 and of the healthy, 13.02 ± 0.88, showing a difference of 0.64 ± 0.10. The ratio of 0.64 to 0.10 is high enough to indicate odds of nearly 20,000 to 1 that this difference is a significant one. If the mean thickness of the healthy leaves for 1930 be represented by 100, that of the leaves from the giant-hill plants will be as 95.08. For further comparison see figures 2 and 3. For the 1932 material, if the mean thickness of the healthy be represented by 100, that of the leaves from the giant-hill will be as 87.

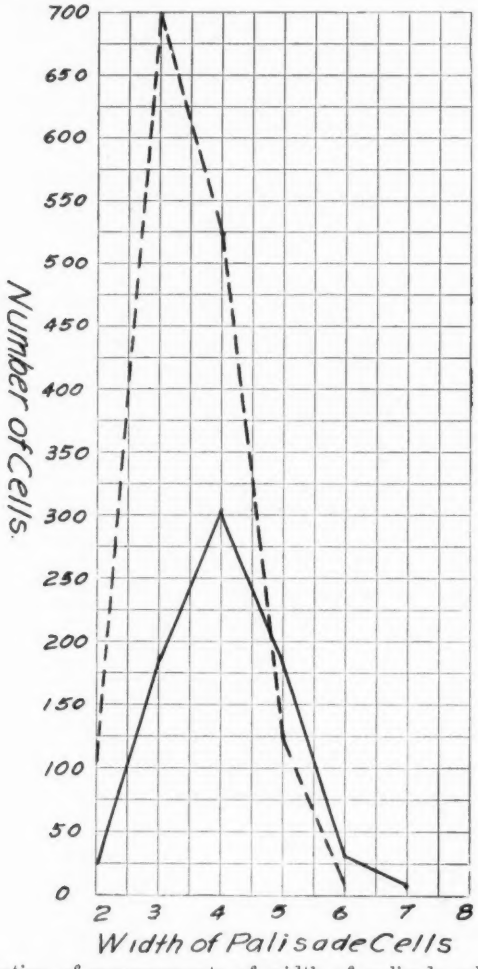

FIG. 4. Distribution of measurements of width of palisade cells, in terms of micrometer spaces. One space = 0.004 mm. Solid line for healthy, broken line for giant-hill plants.

The analysis of the material secured and studied in 1932 confirms these results, but with even greater statistical significance. In this case the mean of the thickness of giant-hill leaves (16 plants) is 0.181 mm. and that of the healthy (14 plants) is 0.206 mm. In terms of the spaces used, the mean

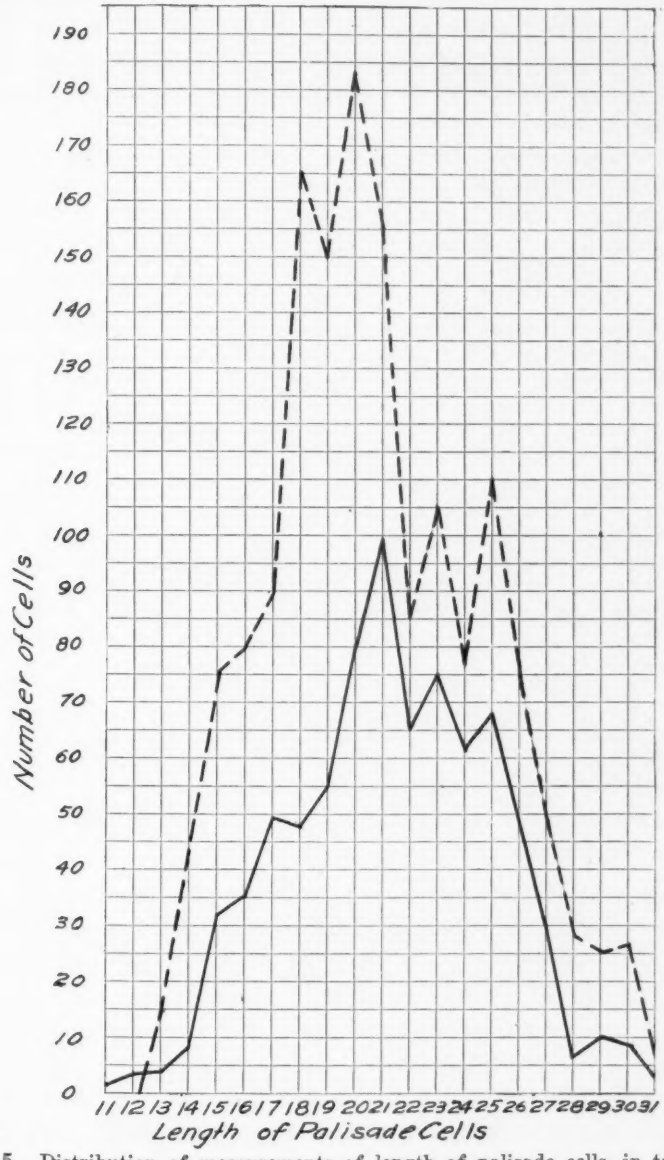

FIG. 5. Distribution of measurements of length of palisade cells, in terms of micrometer spaces. One space = 0.004 mm. Solid line for healthy, broken line for giant-hill plants.

of the giant-hill is 10.38 ± 0.134 and of the healthy 11.93 ± 0.047, showing a difference of 1.55 ± 0.14. The ratio of the difference to its probable error indicates that this trial would need to be repeated more than 1,000,000 times before a difference as great as this, attributable to chance alone, could be found. In view of the fact that these differences in leaf thickness in giant-hill and healthy potato plants are in the same direction and of so great a magnitude in relation to the total leaf thickness, it can be safely affirmed that leaves of giant-hill potato plants are definitely thinner than those of healthy plants.

This difference in leaf thickness led to the suggestion that the palisade mesophyll tissue might also show differences. A study of the cells of the palisade mesophyll tissue from 1930 material confirmed the first impression of such differences. Graphs made of lengths, widths, and ratios of widths to lengths of these cells (Figs. 4, 5, 6) indicated that they showed differ-

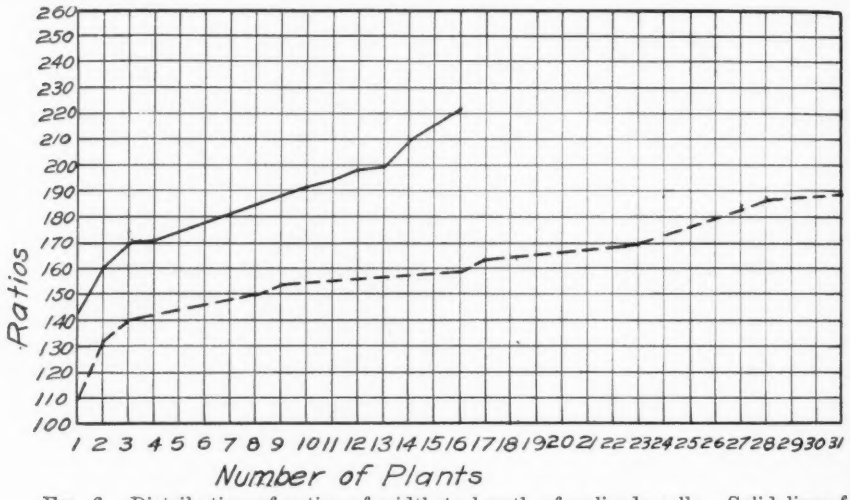

FIG. 6. Distribution of ratios of width to length of palisade cells. Solid lines for healthy, broken line for giant-hill plants.

ences in the diseased and healthy condition. The means of the widths were found to be 0.01584 mm. for the giant-hill and 0.01748 mm. for the healthy; of the lengths 0.085604 mm. for the giant-hill and 0.08644 mm. for the healthy. In terms of spaces these show the following:

Cell dimension	Giant-hill	Healthy	Giant-hill less than healthy by these amounts
Mean width	3.96 ± 0.0123	4.37 ± 0.021	0.41 ± 0.0243
Mean length	21.401 ± 0.0067	21.61 ± 0.00947	0.21 ± 0.011
Width-length	0.37 ± 0.0068	0.64 ± 0.0029	0.27 ± 0.00007

Measurements of lengths and widths of palisade mesophyll cells (Fig. 7) from the 1932 material show differences between giant-hill and healthy plants in the same direction. The means of the widths were found to be 0.01292 mm. for the giant hill and 0.01512 mm. for the healthy plants; of the lengths 0.07140 mm. for the giant hill and 0.08052 mm. for the healthy. In terms of spaces these show the following:

Cell dimension	Giant-hill	Healthy	Giant-hill less than healthy by these amounts
Mean width	3.23 ± 0.04	3.78 ± 0.05	0.55 ± 0.06
Mean length	17.75 ± 0.23	20.13 ± 0.22	2.38 ± 0.31

On the basis of these figures from both the 1930 and the 1932 material, it can be stated with reasonable assurance that the palisade mesophyll cells of the giant-hill plants are considerably narrower and somewhat shorter than those of the healthy.

It is evident from a comparison of the figures for 1930 and 1932 that the latter show the greater differences between giant-hill and healthy leaves, both as to their thickness and the length and width of their palisade cells. 1932 was a year showing even more disastrous effects on potato plants than 1930. These comparisons of diseased and healthy leaves furnish added confirmation for the belief rather generally held that adverse growing conditions emphasize diversities that might normally be less apparent.

An analysis of the relation of diameter and length of palisade cells of the 1930 material also revealed some differences. Since it was impossible to secure the diameter and length of one and the same cell as can be done for the dimensions length and width it was necessary in the calculations to use averages of measurements per leaf and thus to sacrifice some of the apparently greater significance which can attach to results based on the larger numbers from individual cell measurements.

Using the 1930 data, the correlations between lengths and diameter were found to be: Giant-hill plants, 0.55 ± 0.023; healthy plants, 0.28 ± 0.087. Difference, 0.27 ± 0.089.

In the calculations of the means of the diameters from averages per leaf the differences between those of giant-hill and healthy palnts were relatively large, the mean of the giant-hill plants being 0.01612 mm. and that of the healthy 0.01824 mm.

Expressed in terms of micrometer spaces the measurements are as follows:

Cell dimension	Giant-hill	Healthy	Difference
Mean diameter	4.07 ± 0.042	4.50 ± 0.098	0.51 ± 0.01

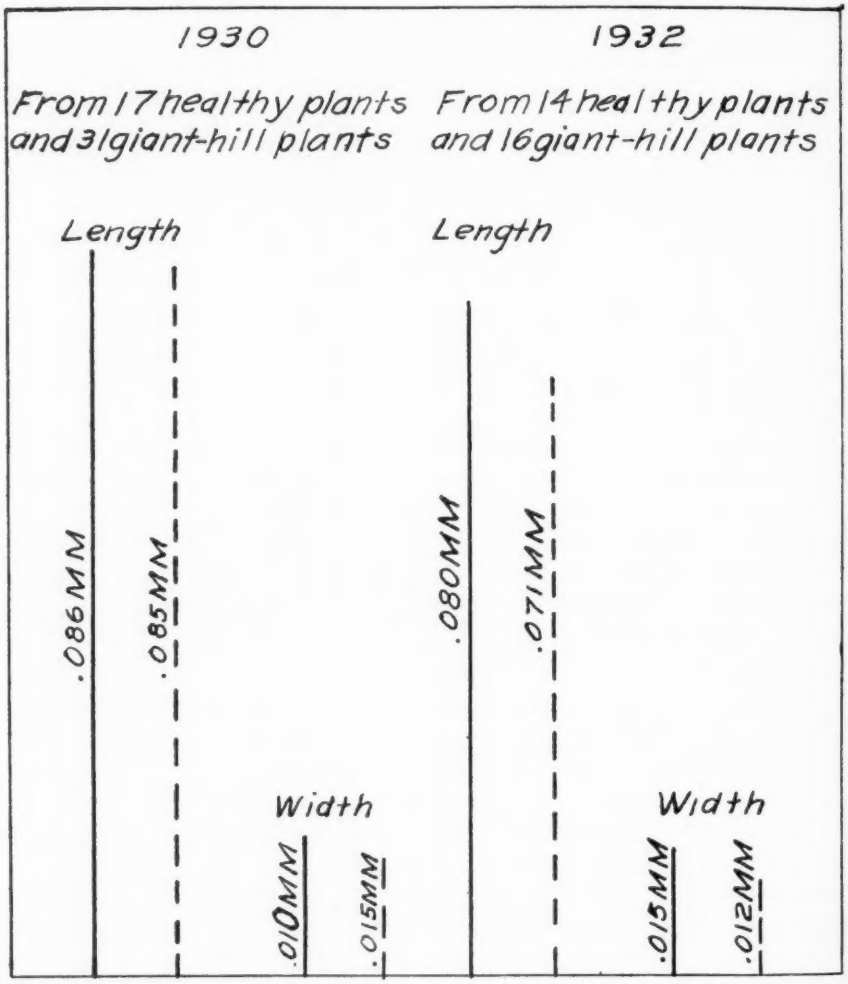

FIG. 7. Comparative measurements of cells of palisade mesophyll expressed in means. Solid line for healthy, broken line for giant-hill plants.

For further comparisons see figure 7.

In the consideration of the differences between the palisade mesophyll cells of the giant-hill and the healthy potato it would appear that those of the giant-hill have a smaller ratio of width to length than the healthy. The

diameters of the giant-hill mesophyll cells are also smaller than the healthy. These differences are indicated further in a comparison of the volumes of these cells calculated on the basis of averages per leaf. Palisade cells from thirty-one plants of giant hill showed a range of volume from 0.0001066 cu. mm. to 0.0002838 cu. mm., with a mean of 0.0001589 cu. mm.; the range for sixteen healthy plants is from 0.0001360 cu. mm. to 0.0003130 cu. mm. with the mean at 0.0002170 cu. mm.

In terms of micrometer spaces the volume calculations for palisade cells are as follows:

Cell dimension	Giant-hill	Healthy	Difference
Mean	248.38 ± 16.34	337.5 ± 22.58	89.12 ± 26.73

The odds are greater than 40 to 1 that this difference is significant. The difference may be otherwise expressed; if the mean volume of palisade cells of healthy leaves be represented by 100, that of giant-hill would be as 73.59.

Counts made of the number of cells to 0.2 mm., the length of the micrometer scale, indicated that there was no significant difference in the number of palisade mesophyll cells per 0.2 mm. in leaves of giant-hill and healthy plants. Since the palisade cells in the leaves of healthy plants were found larger than those of giant-hill, this lack of significant difference in cell number needs an explanation. Other things being equal, one would expect to find a larger number of cells associated with the smaller size in the giant-hill plants. Observation, however, indicates that there is slightly more intercellular space in the palisade region in the leaves of giant-hill than in those of healthy plants, and this harmonizes with the lack of difference in the number of cells. The appearance of the chloroplasts shows no great and uniform difference in the cells of the plants under comparison, though there may be an indication that those in the palisade cells of the giant-hill are somewhat less plump and round than those of the healthy leaves. It is of interest to note that the starch grains are associated with the chloroplasts, either actually within the chloroplasts or on their surface.

A comparison of measurements and leaf counts of 9 healthy and 7 giant-hill plants reveals differences that harmonize with field descriptions of these plants. The top portions of these plants, including 5 nodes with their attached leaves, show a mean of 32.0 ± 1.3 cm. total length for the healthy and 25.28 ± 0.51 cm. for the giant-hill, a difference of 6.72 ± 1.39. This is a definite expression of the "constriction of the tops" observed above. The tips of these same stems, on the other hand, showed a mean of 9.0 ± 0.62 leaves for the healthy and 4.85 ± 0.41 for the giant-hill plants, a difference of 4.15 ± 0.74.

The leaves of giant-hill plants show comparatively few macroscopic features comparable to those of potato plants affected with various other viroses. Folsom *et al.* (11), however, mention slight chlorosis as a characteristic, while Lauer (18) emphasizes the dark green of the leaves. Several writers (6), (31), refer to slight rolling of the margins. In studies of tomato and tobacco leaves (26), (27), and potato leaves (28) affected with mosaic, various modifications of the palisade tissue have been reported. Salaman and Bawden (29) state that it is "probable that pathological conditions of the leaf lead to phloem necrosis." In view of this suggestion, it seems pertinent to add the further suggestion that in giant hill plants, where the leaves show definite histological differences from the normal and where there is certainly some disturbance of the phloem relations in the stem, we have the possibility of a relationship between these two features in leaves and stems. Potato plants affected with giant hill, in regions where the disease is important, show a reduced storage of carbohydrates in their lessened tuber production. It is perhaps not too presumptuous to suggest that there may be a relation between this reduction of carbohydrates in the tubers and the disturbance in palisade mesophyll cells, which is indicated in the analysis of their measurements here presented.

A careful study of the leaf tissues showed no such cell inclusions as are reported by Hoggan (16) to be invariably present in the chlorotic areas of tobacco leaves affected with mosaic. The technique employed may have failed to reveal them. But Hoggan also reports the absence of such inclusions in the cases of 8 other viroses.

The measurements of the petioles of giant-hill and healthy plants from 1930 material show that in the former the inner phloem is slightly more extensive than in the healthy plants, but not significantly so. The xylem also appears to be more extensive in the petioles of giant-hill than in those of healthy plants. The outer phloem, on the other hand, is somewhat less extensive in the former than in the latter. The mean of the xylem in petioles of giant-hill plants is 0.1614 mm. and of the xylem of healthy plants is 0.1389 mm. The mean of the inner phloem is 0.1604 mm. in giant-hill and 0.1508 mm. in the healthy plants. Expressed in micrometer spaces these measurements are as follows:

Thickness in cross section	Giant-hill	Healthy	Increment Giant-hill over healthy
Mean xylem	9.20 ± 0.11	$8.34 \pm .010$	0.85 ± 0.15
Mean inner phloem	9.23 ± 0.18	8.62 ± 0.17	0.61 ± 0.25
γ Xylem and phloem	0.45 ± 0.016	0.28 ± 0.0097	0.16 ± 0.018

The comparison of the correlations between xylem and inner phloem in the petioles shows that both the xylem and the inner phloem appear to be slightly greater in amount in the giant-hill than in the healthy plants and that the correlation between xylem and phloem is significantly higher in the diseased than in the healthy plants.

If the inner phloem in the petioles of healthy plants be represented by 100, that in petioles of giant-hill plants would be as 107.07. The odds, however, are very low that this is a significant difference. If the xylem in the petioles of healthy plants be represented by 100, that in petioles of giant-hill plants would be as 116. If the outer phloem in the petioles of healthy plants be represented by 100, that in petioles of giant-hill plants would be as 94.5. For further comparisons, see figure 8.

This situation in the petioles is of particular interest in connection with

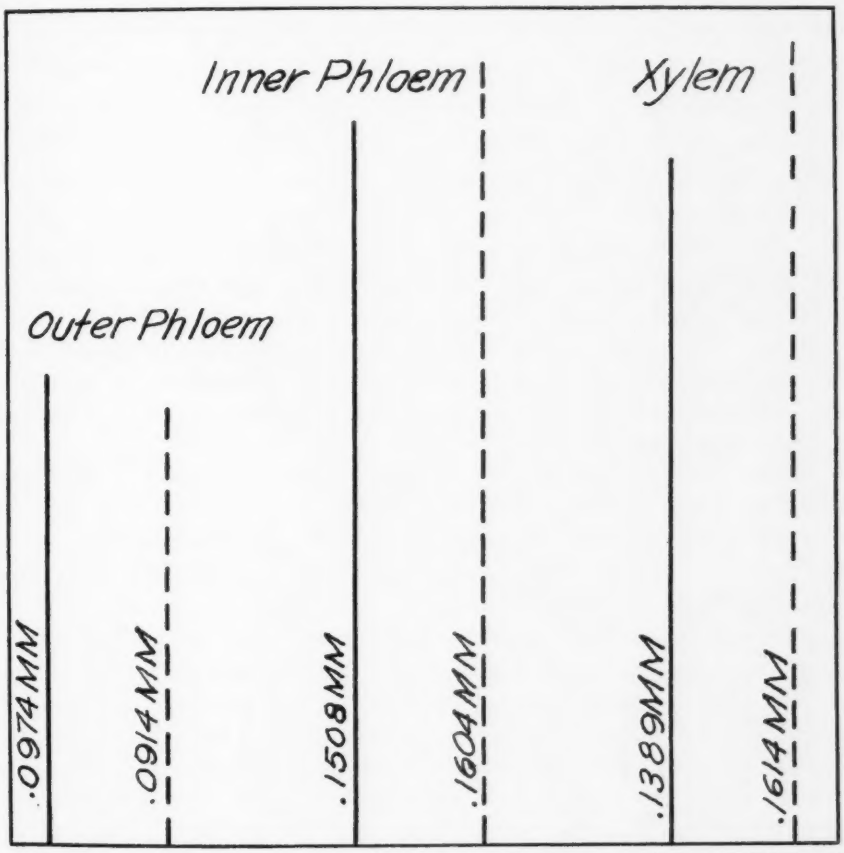

FIG. 8. Comparative measurements of xylem and phloem in petioles. Solid line for healthy, broken line for giant-hill plants.

that found in the stems. Similar comparative measurements in stems show that the inner phloem in the stems of healthy plants is significantly of greater extent than in the giant-hill plants. Computed for the 1930 material, the means of the outer phloem are 0.1240 mm. in the giant-hill and 0.1345 mm. in the healthy; of the xylem, 0.3046 mm. in the giant-hill and 0.3178 mm. in the healthy; of the inner phloem 0.3666 mm. in the giant-hill and 0.4782 mm. in the healthy. The measurements were made on 17 healthy and on 31 giant-hill plants.

In terms of micrometer spaces these tissues are measured as follows:

	Giant-hill	Healthy	Increment giant-hill over healthy
Mean outer phloem	7.08 ± 0.92	7.74 ± 0.15	-0.65 ± 0.93
Mean xylem	17.41 ± 0.26	18.16 ± 0.26	-0.75 ± 0.37
γ Outer phloem and xylem	0.20 ± 0.036	0.15 ± 0.051	0.09 ± 0.06
Mean inner phloem	20.95 ± 0.28	27.33 ± 0.42	-6.38 ± 0.504
Mean xylem	17.39 ± 0.26	18.38 ± 0.22	-0.99 ± 0.89
γ Inner phloem and xylem	0.18 ± 0.038	0.49 ± 0.01	0.30 ± 0.042

Not only is the inner phloem of the healthy stems of greater extent than that of the giant hill, but the xylem also is very slightly greater and the correlation with the xylem, too, is greater.

If the inner phloem in the stems of healthy plants be represented by 100, that in stems of giant hill plants would be as 76.65. The odds that this difference is significant are greater than 20,000 to 1. For further comparisons see figure 9.

The analysis of measurements made of xylem and phloem in stem sections from the 1932 material shows similarly that the xylem and both the inner and outer phloem, as measured radially, are less extensive in the giant-hill than in the healthy plants. In 1932, stem measurements were made from 2 comparable regions, the second and third internodes from the top. For the second internode, the means of the outer phloem are 0.07995 mm. for the giant-hill and 0.085225 mm. for the healthy; of the xylem, 0.235550 mm. for the giant-hill and 0.280900 mm. for the healthy; of the inner phloem 0.258475 mm. for the giant-hill, and 0.3165 mm. for the healthy. For the third internode, the means of the outer phloem are 0.096775 mm. for the giant-hill and 0.1001 mm. for the healthy; of the xylem 0.3787 mm. for the giant-hill and 0.387275 for the healthy; of the inner phloem, 0.3556 mm. for the giant-hill and 0.403725 mm. for the healthy plants.

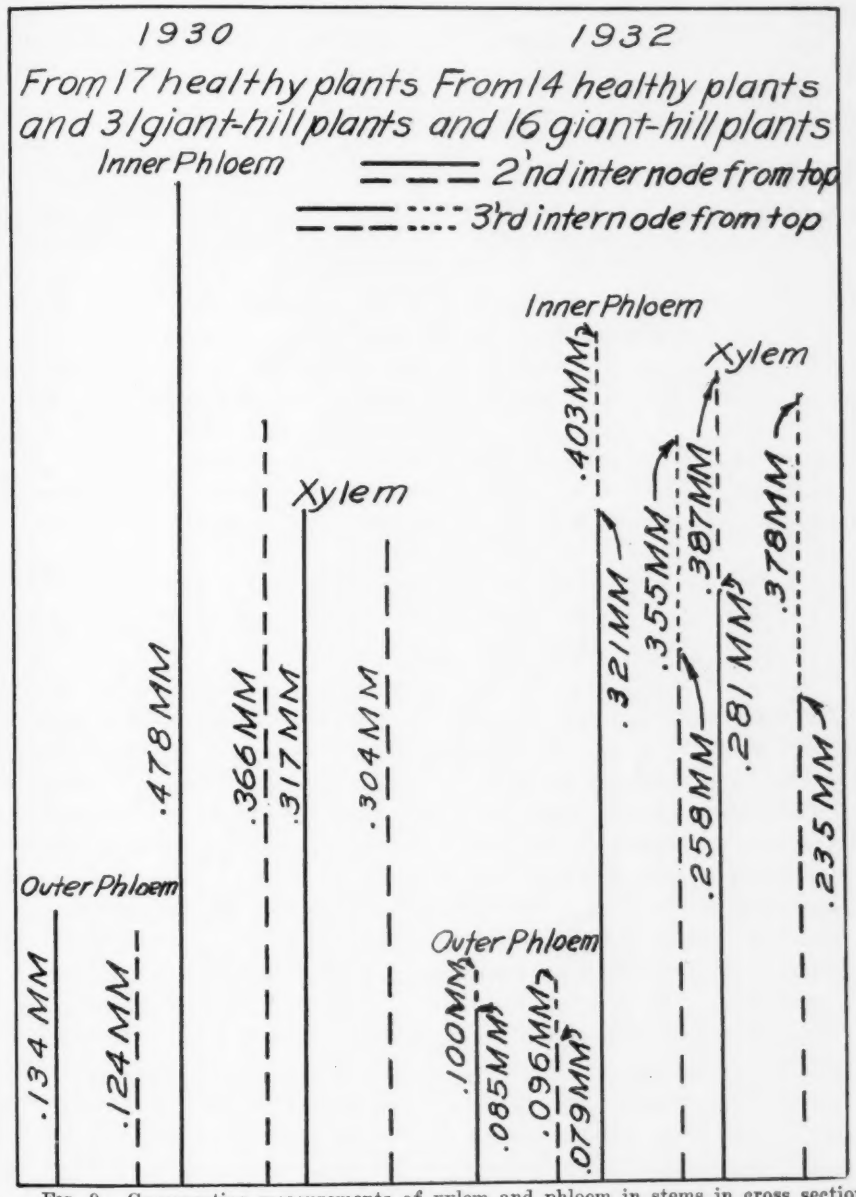

FIG. 9. Comparative measurements of xylem and phloem in stems in cross section expressed in means. Solid line for healthy, broken line for giant-hill plants.

In terms of micrometer spaces, these tissues in the 1932 material show the following measurements:

Second Internode from the Top

	Giant-hill	Healthy	Increment giant-hill over healthy
Mean outer phloem	4.74 ± 0.09	4.87 ± 0.07	− 0.13 ± 0.11
Mean xylem	13.46 ± 0.56	16.04 ± 0.42	− 2.58 ± 0.7 (odds 60 + to 1)
Mean inner phloem	14.77 ± 0.28	18.38 ± 0.38	− 3.61 ± 0.47 (odds over 400,000 to 1)

Third Internode from the Top

	Giant-hill	Healthy	Increment giant-hill over healthy
Mean outer phloem	5.53 ± 0.10	5.72 ± 0.13	− 0.19 ± 0.16
Mean xylem	21.64 ± 0.52	22.13 ± 0.53	− 0.49 ± 0.74
Mean inner phloem	20.32 ± 0.31	23.07 ± 0.65	− 2.75 ± 0.72 (odds 95 + to 1)

The differences shown, particularly by the inner phloem in the 1930 material, are again evident in the 1932 material by odds well above those considered necessary for reasonable assurance. The differences in the outer phloem are statistically insignificant and the xylem shows significant differences in the second but not in the third internode. It is possible that the greater differences shown by the phloem from the second internode from the top over those from the third may be related to the more striking differences in macroscopic features exhibited at the tops of the plants.

Both the petioles and the stems of the giant-hill plants thus show differences from the healthy. The present investigation shows a slightly increased amount of phloem in the petioles or giant-hill plants over that in the normal and a greatly decreased amount of phloem in the stems.

Studies on related viroses (25), (27), (17), (2), (3) have shown various disorganization effects both in the palisade mesophyll of the leaves and in the phloem, especially of the stem. In the case of most of these other diseases the disastrous effects on the host plants are much more readily seen than in giant hill. It is of interest in this connection that giant hill, though causing less conspicuously harmful effects on the potato plants than some of the related viroses, appears to show histological effects, which, though also less conspicuous, are nevertheless similar in their nature to those of these other viroses. The cell activity concerned in photosynthesis must be disturbed where the tissues involved are reduced, as is the case in the leaves in giant-hill plants. And the conducting tissues, though showing no easily observed necrotic modifications, as reported in some of the studies

mentioned above, exhibit variations that must doubtless have associated with them certain physiological disturbances.

An intensive study of the cellular elements comprising the phloem and xylem failed to show any differences between the giant-hill and the healthy plants in these tissues. In view of this absence of differences in the cells it is particularly interesting to note tendencies toward disturbances in the amounts of these tissues present. Studies such as those of Artschwager (2, 3) and Thung (32) of the vascular tissues in stems of potatoes affected with supposedly related diseases show rather definite disorganization of the phloem. Perhaps we have here in giant hill, regarded by Nixon[5] as the precursor of leaf roll, a condition in which phloem disturbance, though not advanced to the stages of necrosis observed in the striking cases of leaf roll, is, nevertheless, present to the extent of showing changes from the healthy condition.

TABLE 1.—*Summary of difference between giant-hill and healthy-potato plants*

Cells of Palisade Mesophyll

1930

	Length	Width	Diameter	Ratio W.L.	Volume
Healthy	21.61 ± 0.009	4.37 ± 0.021	4.58 ± 0.098	0.182 ± 0.003	337.5 ± 22.58
Giant hill	21.4 ± 0.006	3.96 ± 0.01	4.07 ± 0.042	0.156 ± 0.002	248.38 ± 10.29
Difference	0.21 ± 0.01	0.41 ± 0.02	0.51 ± 0.01	0.026 ± 0.0036	89.12 ± 26.73
	odds greater than 1,000,000 to 1			odds nearly 500,000 to 1	odds greater than 40 to 1

1932

	Length	Width
Healthy	20.13 ± 0.22	3.78 ± 0.05
Giant-hill	17.75 ± 0.23	3.23 ± 0.04
Difference	2.38 ± 0.31	0.55 ± 0.06
	odds over 400,000 to 1	odds greatly over 1,000,000 to 1

Measurements made under high power, expressed in terms of micrometer spaces. 1 space = 0.004 mm.

Leaf Thickness

Mean	1930	1932
Healthy	13.02 ± 0.08	11.93 ± 0.04
Giant-hill	12.38 ± 0.06	10.38 ± 0.13
Difference	0.64 ± 0.10	1.55 ± 0.14
	odds nearly 20,000 to 1	odds greatly over 1,000,000 to 1

Measurements made under low power, expressed in terms of micrometer spaces. 1 space = 0.0175 mm.

[5] *Loc. cit.*

Tissues in Petioles

		Outer phloem	Inner phloem	Xylem
Mean	Healthy	5.62 ± 0.07	8.62 ± 0.17	7.93 ± 0.07
	Giant-hill	5.22 ± 0.06	9.23 ± 0.18	9.19 ± 0.11
	Difference	0.40 ± 0.09 odds about 150 to 1	−0.61 ± 0.25 odds about 9 to 1	−1.26 ± 0.13 odds greater than 1,000,000 to 1

Tissues in Stems

1930

		Outer phloem	Inner phloem	Xylem
Mean	Healthy	7.74 ± 0.15	27.33 ± 0.42	18.16 ± 0.26
	Giant-hill	7.08 ± 0.92	20.95 ± 0.28	17.41 ± 0.26
	Difference	0.66 ± 0.93 insignificant	6.38 ± 0.50 odds vastly over 1,000,000 to 1	0.75 ± 0.37 insignificant

1932

Second Internode from Top

		Outer phloem	Inner phloem	Xylem
Mean	Healthy	4.87 ± 0.07	18.38 ± 0.38	16.04 ± 0.42
	Giant-hill	4.74 ± 0.09	14.77 ± 0.28	13.46 ± 0.56
	Difference	0.13 ± 0.11 practically no significance	3.61 ± 0.47 odds over 400,000 to 1	2.58 ± 0.70 odds 64.79 to 1

Third Internode from Top

		Outer phloem	Inner phloem	Xylem
Mean	Healthy	5.72 ± 0.13	23.07 ± 0.65	22.13 ± 0.53
	Giant-hill	5.53 ± 0.10	20.32 ± 0.31	21.64 ± 0.52
	Difference	0.19 ± 0.16 practically no significance	2.75 ± 0.72 odds 95.15 to 1	0.49 ± 0.74 no significance

Measurements made under low power, expressed in terms of micrometer spaces. 1 space = 0.0175 mm.

SUMMARY

Measurements of stem lengths and leaf counts confirm the field description attributing greater constriction of the tops to giant-hill than to healthy potato plants.

The leaves of potato plants affected with giant hill were found to be thinner than those of the healthy plants.

The cells of the palisade mesophyll of leaves of potato plants affected with giant hill were smaller than those of the healthy plants, with a smaller ratio of width to length and smaller volume.

The inner phloem in the petioles of giant-hill plants was found to be slightly greater than that of the petioles of healthy plants and somewhat greater in relation to the xylem than in the healthy plants. The outer phloem was found to be somewhat less in the giant-hill plants.

The inner phloem in the stems of giant-hill plants was found to be considerably less than in the healthy plants and less extensive in relation to the xylem than in the healthy stems.

It is suggested that these disorganizations in photosynthetic and conducting tissues of the giant-hill plants are of related order though of less degree than the disorganizations reported for plants affected with some other virus diseases.

BOX 286, STATE COLLEGE, PENNSYLVANIA

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CYTOLOGY OF PLANT TISSUES AFFECTED BY VIROSES

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Few viruses cause local necrotic lesions. Few even cause the cells to die where they are inoculated. Most viruses when inoculated into a leaf do not develop any evident response at the inoculation point; neither does the inoculation induce evident changes in already differentiated tissue. But all tissues that later develop from a growing point of the inoculated plant may show cytological (12, 29, 30, 31, 33, 34, 37, 38, 39, 43, 46), histological, and even anatomical (4, 6, 24, 35, 42, 44) disturbances. Viruses, therefore, should first be considered as interfering with the growth and differentiation of embryonic tissues.

DIFFERENTIATION OF EMBRYONIC TISSUES IN HEALTHY AND VIRUS-INFECTED BUDS

The actively dividing cell synthesizes most of the available soluble nutrient materials into new cytoplasm (assumedly made of molecules wherein proteins and lipoids are intimately combined) and new mitochondria of similar composition (Guilliermond, Mangenot, and Plantefol (26)) as well as new nuclear material. Meristematic cells characteristically are polyhedric in form. Each contains a nucleus that is large in comparison to the size of the cell and is surrounded by the cytoplasm wherein mitochondria show as small rods (Fig. 1, A). Vacuoles are numerous (Went (48)), small, and often elongated. They contain a thick, homogeneous, stable solution that stains vitally as a whole with neutral red or various other basic dyes.

In buds of healthy plants, the cells enlarge rapidly as the vacuoles imbibe water, swell, and fuse. During that period of active growth, mitochondria retain their rod shape and they evidence no physiological activity beyond dividing. As growth slackens, cells differentiate from the anatomical, histological, and physiological points of view.

In vascular tissues, cells forming wood vessels or sieve tubes have been given more attention than the cells in the wood parenchyma or the companion cells in the phloem. These cells, which evidence their physiological activity through the perpetual and striking shifting of their vacuolar material in the cytoplasm and respond readily to pathological stimuli by forming phenolic compounds, deserve careful consideration. Unfortunately, studying the phloem in diseased plants is difficult, phloem elements being transitory (Dufrenoy (14)).

Green tissues are made of cells where a certain proportion of the mitochondria differentiate into plasts, which, by forming chlorophyll, become

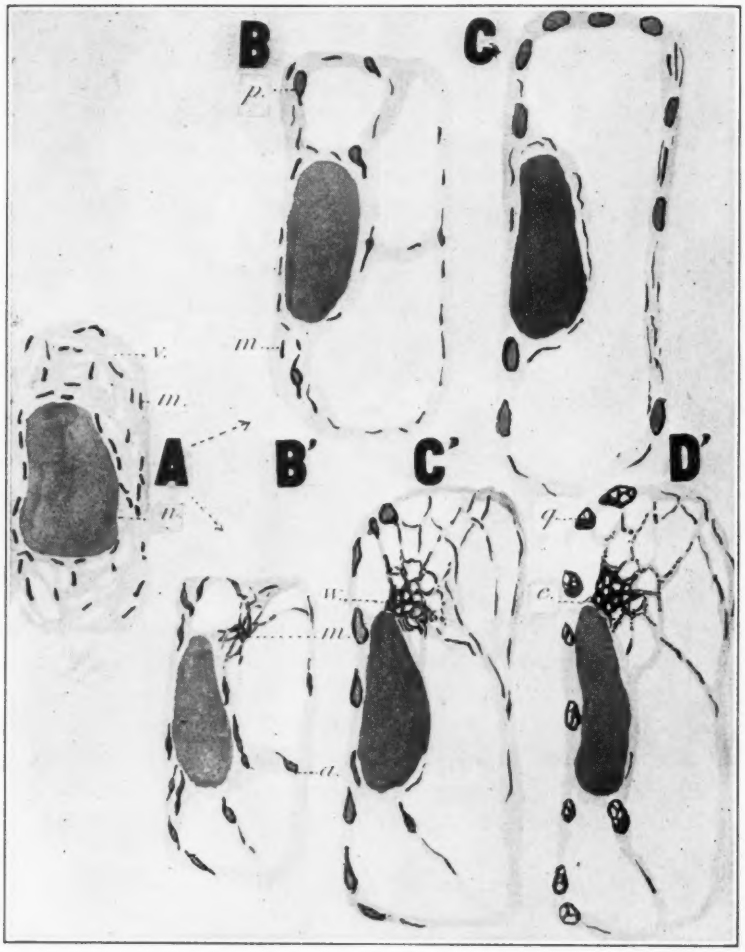


FIG. 1. Effect of virosis on the evolution of the embryonic cell. Starting from the meristematic condition represented in cell A, (with a large nucleus (n) some of the mitochondria (m) should normally differentiate into chloroplasts ((p) B), while the elongated small vacuoles (v) should coalesce and swell into a few large vacuoles (B and C). Under the influence of a virosis, starting from the same meristematic condition A, we see most mitochondria store in starch grains (a), thereby becoming amyloplasts (B′) while some mitochondria (m) clump together along cytoplasmic strands separating a cluster of small vacuoles (w) (B′ and C′). That vacuolated area of the cytoplasm, being highly refringent and staining most deeply, looks like an "inclusion" ((c) D′); plastids disintegrate ((q) D′).

photosynthetically active (Fig. 1, B); they form starch when exposed to light in the presence of CO_2. Cells located deeper, which develop into storage, differentiate some of their mitochondria into amyloplasts, condensing translocated carbohydrates into starch grains.

EFFECT OF VIROSES ON MITOCHONDRIA

What proportion of mitochondria of each cell differentiates into plasts depends on the metabolism. A cell does not manifest many forms of activity at the same time; dividing cells do not store starch; and, conversely, cells crowded with starch do not divide until they have translocated their starch out, in which case the amyloplasts revert to their original condition of mitochondria (Dufrenoy (13)).

The evolution of a great percentage of mitochondria into starch-storing amyloplasts is the first constant cytological response of the cell to growth inhibition in buds or in root tips of plants affected by virosis (Fig. 1, B) of any dwarfing disease (Dufrenoy (7)).

In meristematic tissues, starch storage is concomitant with decreased respiration. Buds of mosaic-affected tobacco use only 4 cubic millimeters oxygen per milligram dry weight per hour, whereas buds of healthy looking tobacco use nearly 6 (Dufrenoy (16)). Plastids may develop in excessive numbers in virus-affected tissues where normally few, if any, would develop. Esau (22) found more "sieve-tube plastids" in the beets affected by curly top than in normal beets. C. F. Lackey and we found that the virus of curly top induces early differentiation of mitochondria into amyloplasts, in post-meristematic cells, and on the contrary, inhibits the differentiation of the "starch sheath" around vascular tissues.

HYPERTONIC DISEASES

Inefficient utilization of the products of photosynthetic activity may be responsible for both leptonecrosis and for the "high pressure developed by the abnormal accumulation of the sugars" (Carsner and Stahl (4)) in the tops of beets affected by curly top, in tomatoes affected by yellows, and in potatoes affected by leaf roll, where starch, photosynthesized in the chloroplasts, can no longer be translocated away, while many other unutilized products (phytosterol) are also stored in the vacuoles.

Vascular tissues may accumulate sugars, due to viroses, such as beet curly top (Carsner and Stahl (4)), or following the deficiency of some element such as boron (Haas and Klotz (28)) when starch translocation is inhibited.

Mosaic.—Early evolution of mitochondria into amyloplasts in virus-infected buds may leave few mitochondria available to develop into chloroplasts in leaf primordia. Many that begin to differentiate may fail to become photosynthetically active. Moreover, such chloroplasts as have differentiated may disintegrate. Starch grains in the plasts are the source of carbohydrate that the cells draw on. Starch usually is translocated out of the chloroplasts during the night, but early in the day the insolated chloroplasts of green cells are again replete with starch grains. In mosaic-

affected cells, even after a period of illumination, the starch grains are thin, elongated, and surrounded by some less condensed carbohydrate, which does not show the staining reactions of starch.

But, while the cell seems to draw freely on its reserves of starch and sugars, it can use up only a small fraction of its proteins. Aspects of plasts in virus-affected cells suggest local proteolytic disintegration: a crescent-shape part of the plastid, wherein traces of starch grains are preserved, with few small oil droplets in between, keeps its normal staining reactions (Fig. 1, D and Fig. 2, C) but the greater part of the plast is swollen into a

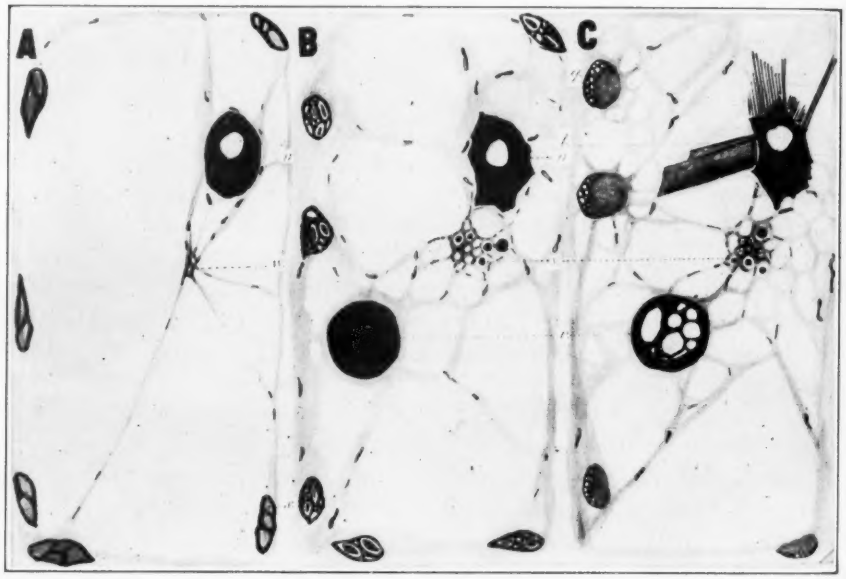


FIG. 2. Effect of a virosis on a palisade cell of *Nicotiana tabacum*. A. Formation of small vacuoles (w); the cytoplasm around becomes highly refringent, the rest of the cell being apparently unaffected and the plastids containing starch grains (s). B. The refringent area of the cytoplasm forms a network of strands, (along which mitochondria are actively dividing) around the small vacuoles (v) within which the vacuolar solution is easily flocculated into precipitates. Other large vacuoles may show vacuolar inclusions (r). Starch (s) is being translocated out of plastids, which assume a vesiculated appearance. C. Amino acids (mostly tyrosine (t) and leucine (l)) crystallize within some part of the cytoplasm. Plastids (q), almost entirely depleted of starch, undergo a partial disintegration and swell. The nucleus (n) becomes distorted.

dimly outlined mass, staining chiefly with erythrosin. This swelling of plasts can easily be watched by mounting in the Ringer solution some pieces of epidermis stripped off the margin of discolored area of iris leaves or of gladiolus flowers. Plasts swell in virus-infected cells of discolored areas much quicker than in neighboring cells from less altered areas, due probably

to a greater abundance of free hygrophilous lipoids. Swollen plastids generally show a green, crescent-shape portion, wherein small oil droplets show brightly, and a diffuse swollen gel mass; brownian movements have been described (Beauverie (1); Dufrenoy (8 and 9)).

ACCELERATED RESPIRATION OF GREEN CELLS AFFECTED BY MOSAIC

Mosaic diseases so impair photosynthesis that cells develop a hypotonic condition as the vacuolar solution is depleted of sugars. Cells depend for their metabolism on the splitting of previously photosynthesized materials or on the translocation of water-soluble material from neighboring cells. In these, as in the starved cells studied by Reed (40), "the kinetic energy necessary to carry this process must be generated by respiration."

Using Warburg apparatus, we found, with Genevois (25), that light green areas of mosaic leaves use more than 4 cubic millimeters of oxygen per mg. of dry weight per hour, while dark green areas of the same leaves use less than 4 and green tissues of healthy leaves use about 3. A respiratory activity as high as $I_{O_2} = 4$ was found in leaves that had not yet developed external symptoms of mosaic, but in which the cells already showed the fine vacuolization characteristic of mosaic.

The higher respiratory intensity in mosaic cells is associated with a distribution of the vacuolar material between numerous small vacuoles, as contrasted with its presence in one large central vacuole in the healthy cell. Numerous narrow vacuoles show in the cytoplasm of epidermal cells of *Nicotiana glutinosa* 24 hours after the virus from *N. tabacum* has been rubbed on the leaf. In that exceptional case, general vacuolization is soon followed by the collapse of the cells. Necrotic lesions appear where the virus has penetrated, and check its generalization. Conversely, viruses that become systemic kill few, if any, cells. Most infected cells survive long enough to respond first by localized vacuolization and next by further localized biochemical changes, causing the formation of the cell inclusions.

THE CYTOPLASM VACUOLE INTERFACE

The living processes may be considered to take place mainly along the surfaces of contact between cytoplasm and vacuoles; local alteration of the cytoplasm, featuring "inclusions," may result from the breaking down of the adjustment of the two phases in contact along the interphase. (Fig. 1, B′ to D′). Much may therefore be grasped from the study of contact surfaces between cytoplasm and vacuolar material in living cells from leaf epidermis of tobacco, iris, or hemerocallis, or flowers of gladiolus, which are transparent enough for their contents to be studied under the highest magnification of the microscope or of the ultramicroscope (Dufrenoy (17)). Neither the cytoplasm itself nor the vacuolar contents of a living cell are

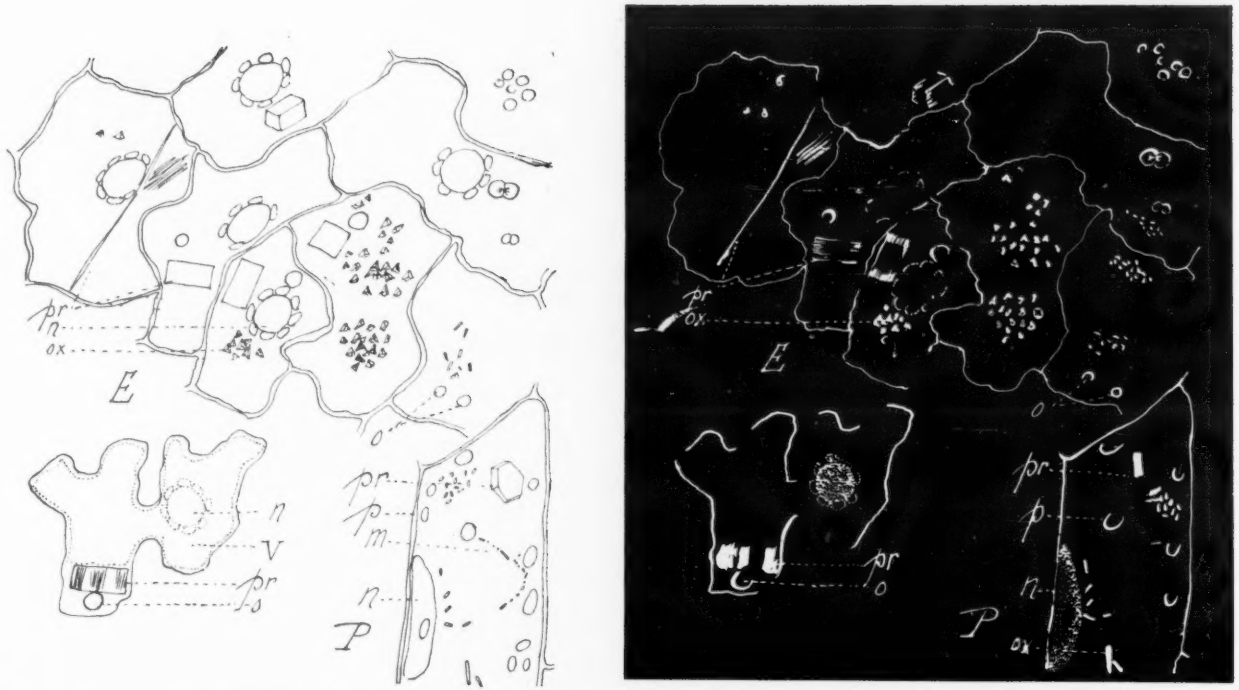


FIG. 3. Microscopic and ultramicroscopic aspects of (E) epidermal, and (P) palisade cells of mosaic leaf of *Nicotiana tabacum*. (n) nucleus; (p) plasts; (pr) tyrosin crystals (striated bodies); (o) vacuolar inclusions (possibly mixtures of lecithin and sterols; (ox) calcium oxalate crystals within the vacuoles (v).

visible under the ultramicroscope. The flowing of the cytoplasm between vacuoles is made evident through the bright oil globules that it carries along, but the cytoplasmic matter itself is optically empty; therefore, only the contour of mitochondria or plastids is illuminated out of the dark field. The

FIG. 4. Living hair cell from chlorotic area of mosaic leaf of *Nicotiana tabacum*, as seen under dark-field illumination. (*n*). Nucleus showing dimly. The separation between some cytoplasmic strands and vacuoles shows brightly. Normal cytoplasm itself is dark, but the included plastids (*p*) show from their bright contours. Fat globules (*l*) are brightly illuminated. Where proteolytic processes are active, dark cytoplasm is being gradually altered into bright proteic crystals (c). ×2000.

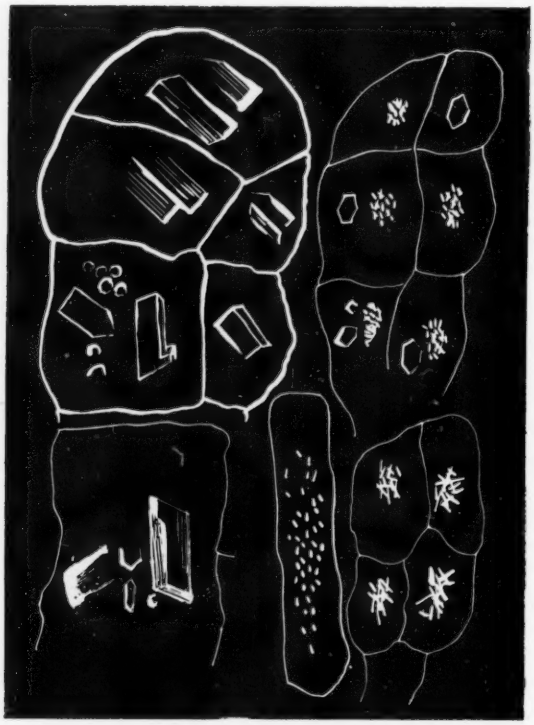


FIG. 5. Cells of hair of mosaic tobacco leaf seen under the ultramicroscope.

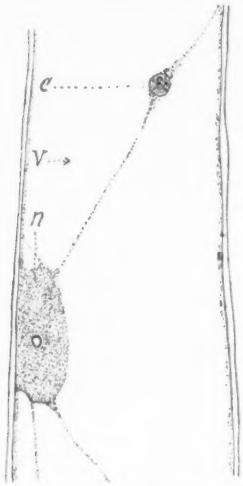


FIG. 6. Beginning of the formation of a vacuolated (*c*) inclusion being shifted along the cytoplasmic film between vacuoles (*v*) in a cell of a leaf hair of tobacco affected by mosaic; (*n*) is the nucleus.

vacuolar solution also is optically empty but for crystals and, eventually, some vacuolar inclusions that may float in it.

Figures 3, 4, and 5 show the sandy crystals of calcium oxalate brightly illuminated within the dark vacuole solution. The interphase between the cytoplasm and the vacuolar material usually does not show on the dark field, so that we know of the cytoplasmic film only from the flow of the bright oil droplets carried along cytoplasmic currents. However, the modifications

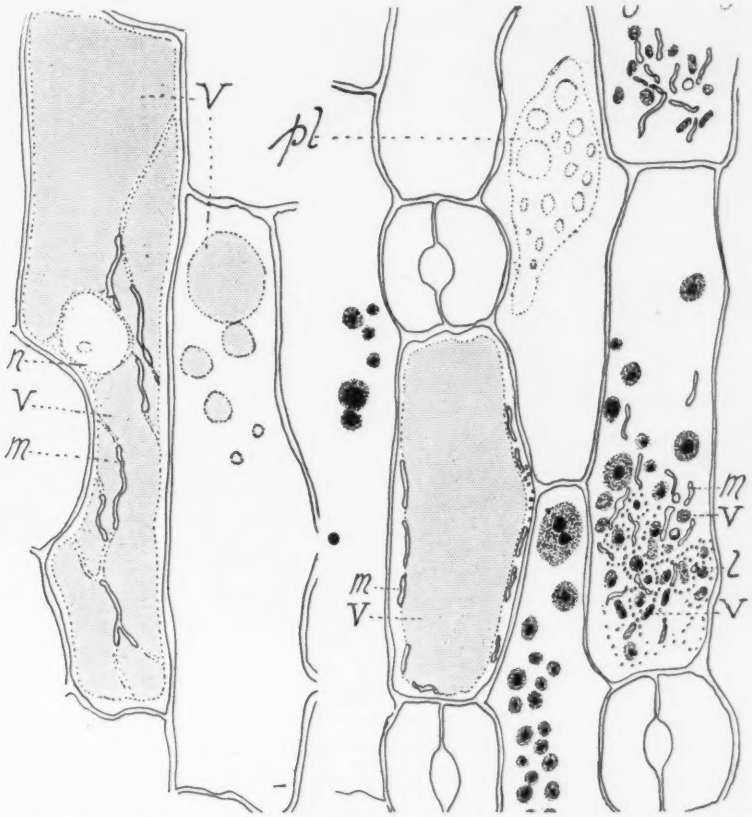


FIG. 7. Piece of living epidermis stripped from chlorotic area of leaves of *Hemerocallis* sp. stained in isotonic neutral-red solution. Normal cells show one or a few large vacuoles (*v*) surrounded by cytoplasmic strands where mitochondria (*m*) show as bright threads. Affected cells show a cluster of small vacuoles (*v*) within a network of cytoplasmic strands where short mitochrondria are densely interwoven and refractive fat globules (*l*) are numerous. The vacuolar solution of large vacuoles stains pink, as a whole, with neutral red and is shown by stippling; that of the small vacuoles is partially precipitated into deeply staining intravacuolar spherical bodies (shown black). Necrotic vacuolated cytoplasmic bodies (*pl*), where the vacuolar contents can no longer be stained by vital dyes, are seen in some cells. × 800.

brought about by the virus may result in the cytoplasm vacuoles' interphase becoming bright. This shows in the leaf hair of *Nicotianum tabacum* (Fig. 4) where a bright interface outlines the small clustered vacuoles. The cytoplasm, normally optically empty, gradually becomes bright where it is altered into bright crystals of needle-like tyrosin or plate-like leucine. Tyrosin crystals show also as striated bodies in glandular cells at the tip of a leaf hair (Fig. 5). (Dufrenoy *et al.* 19 and 20.)

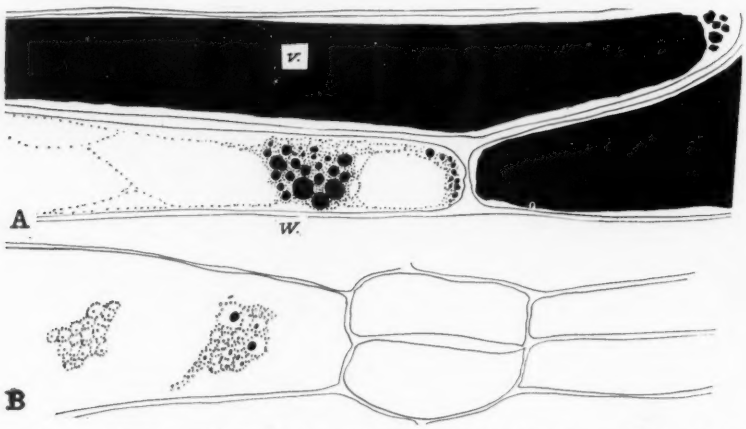


FIG. 8. A. Piece of epidermis of mosaic leaf of *Iris tectorum*. Vital staining with neutral red demonstrates the large central vacuole (*v*) in cells covering green areas and the clustered small vacuoles (*w*) in a cell at the margin of a discolored fleck (vacuolar content shown black). The vacuoles (*w*) are outlined by the numerous oil droplets present in the cytoplasmic films between the small vacuoles. × 800. B. Epidermal cell of badly discolored fleck on *Iris germanica;* the contour of the small clustered vacuoles is outlined by the rows of oil droplets in cytoplasmic strands. The vacuolar solution does not stain as such with neutral red, but is precipitated as vacuolar precipitates (shown black). × 800.

LOCAL MODIFICATION IN THE CYTOPLASM

The gradual building up of a vacuolated "inclusion" through the clumping together of mitochondria in between clustered vacuoles is best observed in hairs of tobacco (Fig. 6) or in the large epidermal cells of iris or hemerocallis leaves or in those of gladiolus flowers. Pieces of epidermis stripped off the margin of a discolored fleck on mosaic hemerocallis (Fig. 7) or iris leaf (Fig. 8 and 9) show that the epidermal cells covering the green area are mainly occupied by one or a few large vacuoles surrounded by a cytoplasmic film wherein long slender mitochondria are evenly distributed. Cells from discolored areas show a cluster of small vacuoles within a network of refractive cytoplasmic strands wherein oil droplets are numerous, and short mitochondria are densely interwoven. The same patterns,

as are easily made out in the living cell, can be stained after killing with mitochondria technic, as shown in figures 9–12. The refractive indices of

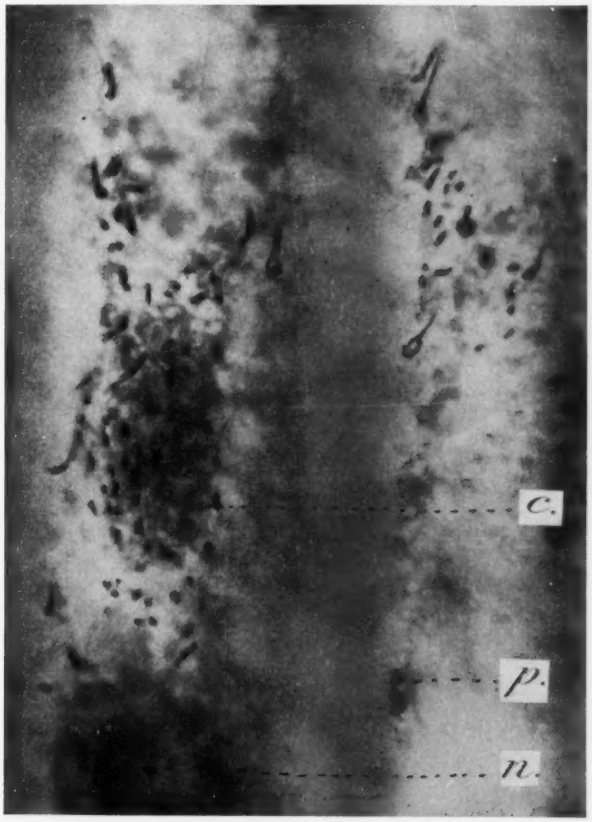


FIG. 9. Left: Epidermal cell of *Iris tingitana* showing an early stage of vacuolated-body formation, where short mitochondria cluster along a network of cytoplasmic trabeculae (*c*), with numerous small vacuoles in between. The nucleus shows at *n*. Right: Part of a cell where mitochondria are evenly distributed; many of these differentiate into plasts (*p*). ×1500.

the "vacuolated cytoplasm" and its crowding with oil droplets (Dufrenoy (8), Clinch (5)) suggest a disintegration of the lipoproteins; a weak solution of eosin in Ringer fluid stains the "vacuolated cytoplasm" and the nucleus red, while the bulk of the cytoplasmic films remain unstained. The stained part of the cytoplasm may be spoken of as dead in contrast to the rest of the cytoplasm, which is still alive. That death may affect part of the cytoplasm in a cell, while the rest remains living, was recorded by Went as early as 1888. Local death in the cell can be observed in tissues starving

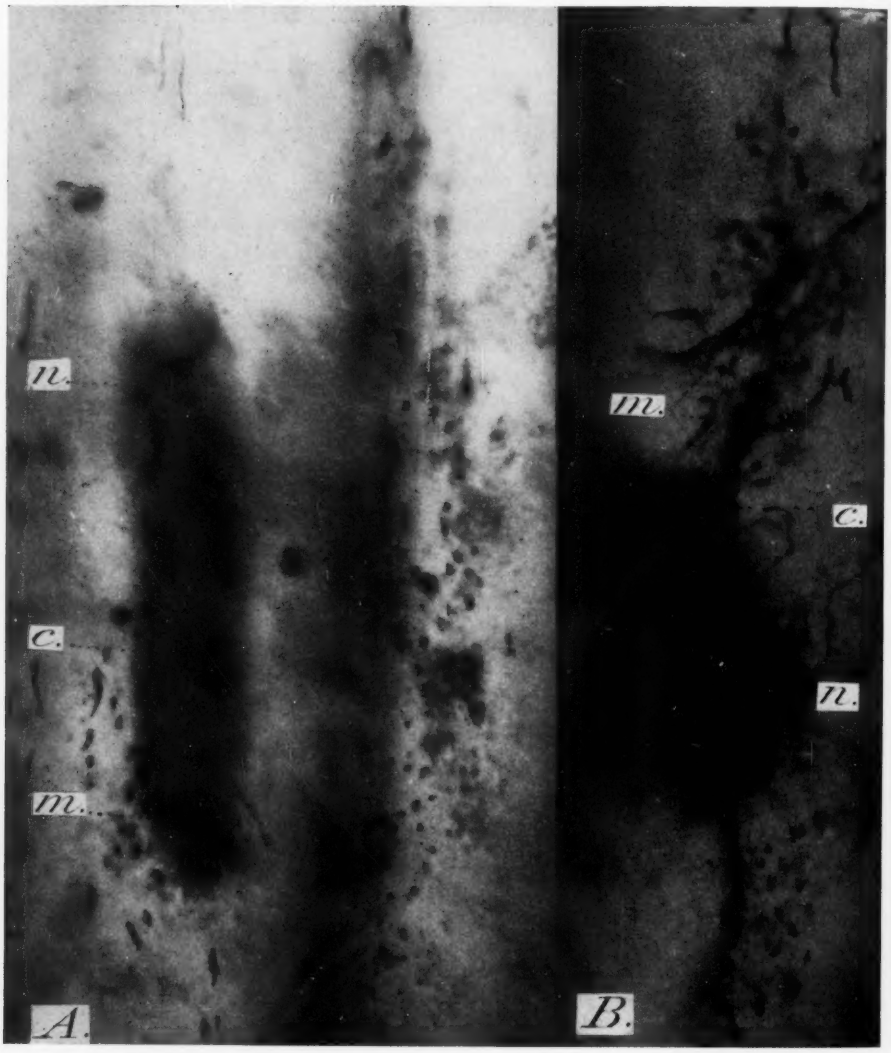


FIG. 10. *A*. Part of two epidermal cells of *Iris tingitana*. Left: Below the nucleus (*n*) a network of cytoplasmic strands stain deeply enough to obscure the mitochondria (*m*) inside; short mitochondria (*m*) can be made out on the border of that deeply staining cytoplasmic area (*c*). Long mitochondria show in the remaining part of the cytoplasm. Right: A cell shows a number of small clusters of short mitochondria which are so many foci for vacuolated-body formation. *B*. Mitochondria (*m*) are densely interwoven along the network of deeply staining cytoplasmic strands (*c*) around the nucleus (*n*). ×1500.

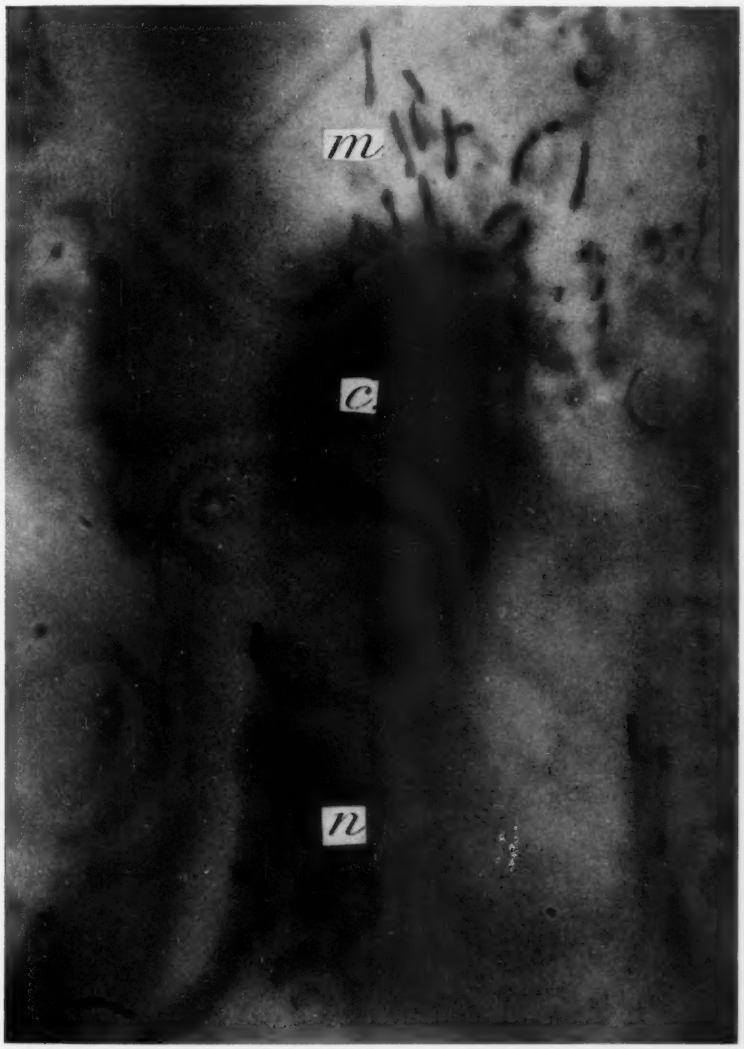


FIG. 11. Above the nucleus (*n*) of an epidermal cell of *Iris tingitana*, a certain cytoplasmic area (*c*) stains so deeply as to show as a vacuolated body with surrounding cytoplasmic strands, where mitochondria are fairly numerous (*m*). ×3000.

under aseptic conditions or grown in the absence of some essential element (Dufrenoy (15, 18)). Sheffield (45) obtained cell inclusions by feeding molybdenum to the tissues. The similarity of localized cytoplasmic response to so many apparently different stimuli suggests a common basic cause, which we assume to be enhanced proteolytic activity. It was recently reported

FIG. 12. Part of epidermal cell of *Iris tingitana* showing the ultimate stage of vacuolated body (*c*); deeply staining cytoplasmic area below the nucleus (*n*) is sharply made out from the remaining cytoplasm, where mitochondria (*m*) have assumed a bead-like appearance through active division. × 1500.

that urea may accumulate in one of the vacuoles of a cell, there to induce active proteolysis of the immediately surrounding cytoplasm.

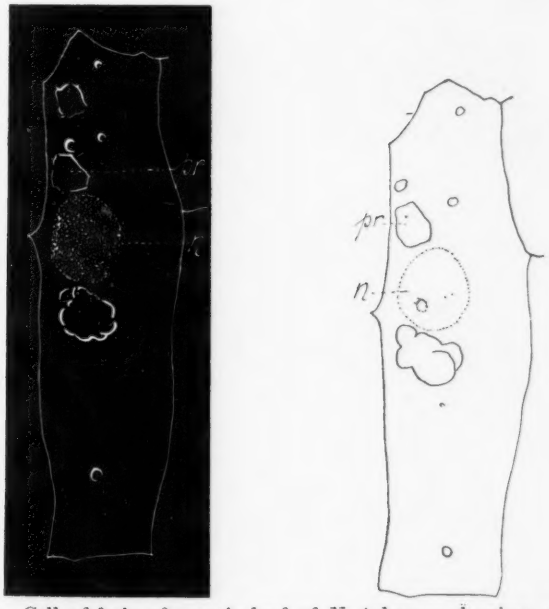
PHYSICAL CONDITION OF THE VACUOLAR SAP

a, pH.—Viruses seem to shift the pH of the vacuolar solution from the more acid towards the less acid or the neutral. Calcium oxalate crystallizes as monoclinic crystals in the vacuoles of healthy *Nicotiana tabacum* or *N. glauca*, where the pH may be 3 or less. It crystallizes as tetrahedrons in mosaic-affected cells, suggesting a pH higher than 5.

Similar evidence is obtained from the purple or blue flecking of red or pink gladioli by mosaic and, better still, by the "breaking." Petals of pink gladioli owe their color to the anthocyanol in solution in the large central vacuole of each epidermal cell. Mosaic causes anthocyanol to crystallize out of its vacuolar solution into a purple spherocrystal, giving the affected epidermis a slaty appearance. Experimental crystallization of that anthocyanol may be obtained by buffering the solution of pink cells to pH 8.

The lowering of acidity by the virus may be correlated to increased rate of respiration, oxidizing further organic acids to CO_2.

b, Stability of the Vacuolar Solution.—In the large vacuoles of epidermal cells of iris or hemerocallis leaves affected by mosaic, the vacuolar solution stains readily as such by neutral red; but it is precipitated as vacuolar precipitates within the small vacuoles that are clustered in the "vacuolated cytoplasm."



FIGS. 13–14. Cell of hair of mosaic leaf of *N. tabacum* showing, under the microscope (right) and the ultramicroscope (left): The nucleus (*n*), a lobed inclusion, and crystals of leucine (*pr*).

c, Specialization of the Vacuoles.—"When a cell contains several vacuoles these may differ as to their contents" (Went (48)). This is especially true of cells affected by virus or other causes of trouble. Vital staining with neutral red or Nile blue, as well as microchemical tests, confirm the coexistence, within the same cell, of vacuoles differing as to the pH and contents of the solution within. Accumulation of phenolic compounds in small clustered vacuoles occurred in epidermis of *Nicotiana tabacum* where we inoculated the "yellow mosaic" virus (Dufrenoy (16)). Ordinary green mosaic induced slower but more complicated changes involving calcium oxalate and presumably phytosterol (Figs. 13 and 14) with other sterids, lecithins, and phosphatids. Cells of *N. tabacum* may contain spherical or vesiculated inclusions (presumably made of mixtures of lecithin and sterols or possibly involving a colloidal form of carbohydrate not only as a result of mosaic diseases, but also when abnormal chromosomic constitution inhibits normal growth or differentiation. These inclusions are to be compared with those described by Reed and Dufrenoy (41) in mottle leaf of Citrus. Such bodies, which have been extensively studied by Guilliermond (27) seem to be common. (Fig. 15, *r.*)

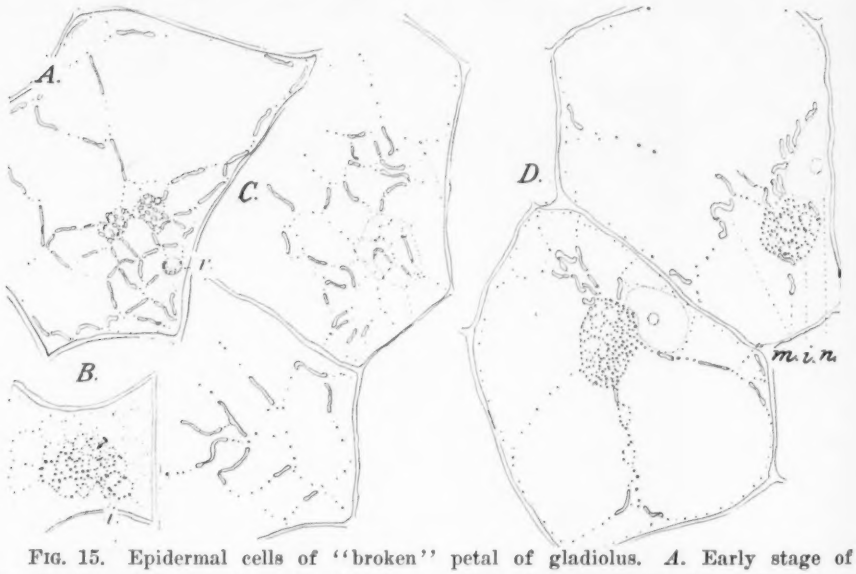


FIG. 15. Epidermal cells of "broken" petal of gladiolus. *A*. Early stage of vacuolization of the cytoplasm, showing the crowding of short mitochondria and oil droplets, and formation of vacuolar inclusion (*r*). *B* demonstrates the oil droplets (*i*), after staining with indophenol blue. *C* and *D* show a later stage of the vacuolization and accumulation of oil droplets (*i*) near the nucleus (*n*). Mitochondria (*m*) are short near the vacuolated cytoplasm, but they retain their normal length on the slender cytoplasmic films separating the large vacuoles. *C* was observed by focusing on the lower level, *D* on the upper level of the same two living cells. ×1500.

THE INTRACELLULAR VIRUS

Squeezing out the contents of animal cells showing inclusions as the result of virus infection made it possible for Borrel (3) and Ledingham (35) to demonstrate, by proper staining, minute granules 0.2 μ in diameter that may represent the virus. Squeezing the cytoplasm from cells under the Meves killing fluid, mordanting with iron tannate and staining with carbolfuchsin, following the Borrel technic, enabled us (17) to obtain photomicrograph (Fig. 16) of similar granules from mosaic cells of tobacco leaves or of

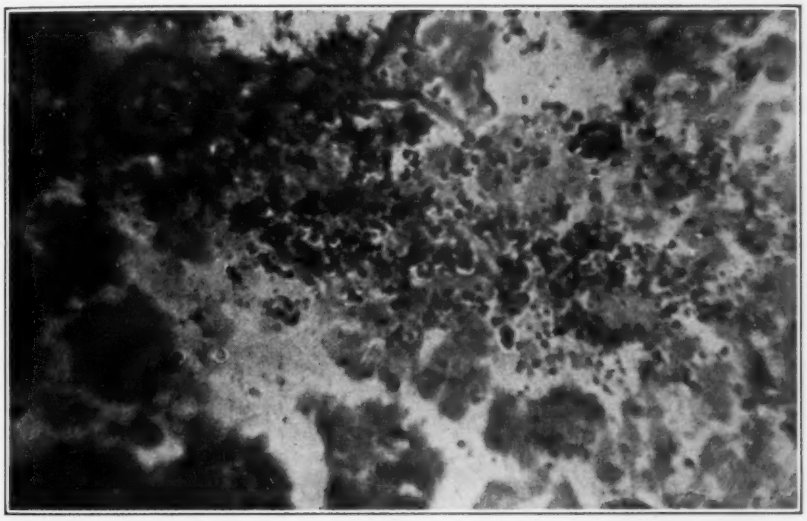


FIG. 16. Photomicrograph of the cytoplasmic film squeezed out of a mosaic epidermal cell of tobacco fixed with the Němec killing fluid, mordanted in iron tannate, and stained with Ziehl's fuchsin. The deeply staining granules showing on the hazy cytoplasmic background may be assumed to represent virus particles, considerably enlarged by a coating of adsorbed iron tannate. × 2000.

"broken" tulip petals. Bewley (2), using a different technic, also sees the virus of tomato mosaic in the form of a granular deposit "with deeply staining fragments." B. M. Duggar (21) recently localized " the main virus concentration" "in the protoplasm" [of tobacco cells] "and not in the cell sap."

RELATION BETWEEN CYTOLOGICAL RESPONSE AND RESISTANCE

When virus infection becomes systemic the response of the cytoplasm in each cell tends to be localized to a small area, honeycombed by the formation of small, clustered vacuoles wherein phenolic compounds may accumulate. That vacuolated part of the cytoplasm may to all appearances die, but the

bulk of the cytoplasm in the cell continues to live, although its metabolism may be altered.

Such a situation is strikingly similar to that resulting from the infection of cells by haustoria of such parasites as develop a systemic infection, without causing necrosis. The cytoplasm around the haustorium may become finely vacuolated—it may be altered to the extent of being, to all appearances, dead; but the greater part of the cytoplasm in the infected cell remains alive, although it may manifest an altered metabolism.

Conversely, when a fungus infection fails to become systemic, but is responsible for such necrotic responses as are associated with resistance, cells in the vicinity of the hyphae collapse before they are ever penetrated by a haustorium, and neighboring cells accumulate phenolic compounds in a large central vacuole. Similar phenomena are observed whenever a virus fails to develop systemic infection, the best example being the early formation of phenolic vacuolar material in cells of *Nicotiana glutinosa* where local

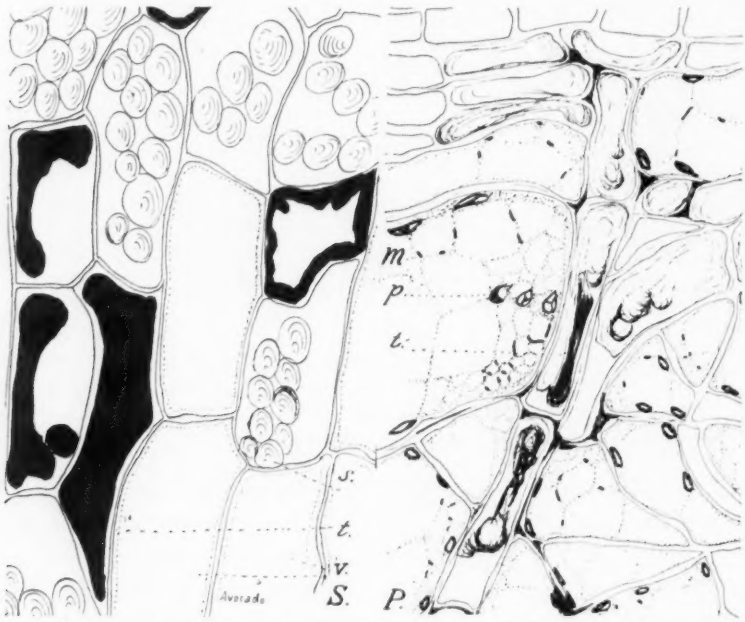


FIG. 17. *S.* Longitudinal section through sun blotch lesion of cortex parenchyma of avocado, showing cells with brown phenolic vacuolar contents (*t*) between living cells, some of which are crowded with starch grains (*s*), while others are occupied mainly by a large vacuole (*v*), the contents of which stain as a whole with neutral red. (Professor Horne's material.) *P.* Transverse section through Citrus leaf showing a lesion of psorosis (Dr. Fawcett's material)—long, slender cells, surrounded by swollen pectic walls, and containing brown phenolic vacuolar precipitates (*t*) are crushed between mesophyll cells, showing plastids (*p*) and mitochondria (*m*).

necrosis develops as the result of infection by the virus of *N. tabacum* mosaic as studied on plants kindly made available by Drs. Takahashi and Shapovalov.

The accumulation of phenolic compounds in a circular layer of cells bordering each lesion is responsible for the "ring-spots" pattern of certain viroses (Dufrenoy (10)). For the definition of ring spots, see K. Smith (47).

The typical "sun-blotch" lesions of avocado (Horne (32)) are characterized by longitudinal series of cortical cells developing large vacuoles full of phenolic compounds; and similar "phenolic" cells are the most conspicuous features of the scaly bark disease of Citrus (Fig. 17), newly recognized as due to a virus (Fawcett (23)).

DISCUSSION

The cytoplasm in the normal living cell looks so homogeneous that neither the microscope nor even the ultramicroscope can reveal anything of its intimate structure. In the homogeneous cytoplasm, mitochondria and eventually plastids (which proceed from mitochondria) show without any staining; vacuoles can easily be demonstrated through the use of vital dyes. We can therefore easily learn through the study of the architecture of the normal cell that a certain distribution and arrangement of mitochondria and vacuoles within the cytoplasm is linked up with the maintenance of life. We can even correlate certain readjustments to certain modifications of the cell metabolism, but we can learn little of the structure of the cytoplasm itself, of mitochondria, or of plastids, through the direct observation of the healthy cell.

Agents that result in the partial breakdown of that homogeneous structure or that interfere with the making up of the cell constituents may reveal some clue as to what materials comprise the cytoplasm and plastids. Viruses are most effective in that respect. Through the study of the virus-infected cell we should learn much about the structure of the healthy cell.

Examination of a great number of living cells from different tissues—some healthy, others diseased—makes it evident that the more active the life processes in the cell (1) the greater the area of interfacial contact between cytoplasm and vacuoles; and (2) the more rapid the changes of relative positions of cytoplasm and vacuoles in the cell.

Be it physiological, as evidenced in glandular cells of the hairs of carnivorous plants (Drosera), or pathological, as in cells affected by microscopic parasites or by viruses, enhanced biochemical activity is evidenced by the division of the bulk of involved cytoplasm into a number of slender films, spreading and forking into the vacuolar material, as so many partitions, which divide a large vacuole into a number of smaller ones. When the

partitioning off is carried to the extreme, the cytoplasm assumes a honeycombed appearance, which has been described in virus-affected cells under various names, but corresponds to the similar structures resulting from local proteolytic activity induced by the penetration of fungus haustoria, or that can even be observed in cells of tissues starving under aseptic conditions.

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THE MODE OF INFECTION OF DIPLOCARPON EARLIANA AND MYCOSPHAERELLA FRAGARIAE

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INTRODUCTION

During the course of an investigation of the influence of seasonal variations on the amount of infection with the strawberry leaf spot (*Mycosphaerella fragariae* (Tul.) Lindau) and scorch (*Diplocarpon earliana* (Ell. & Ev.) Wolf) under Louisiana conditions, an inconsistency in the results obtained from inoculation experiments was noted that could not be accounted for on the basis of environmental factors. It was discovered that this inconsistency was due to the methods used in inoculating the plants. It was noted that when the inoculum was sprayed on the plant by means of an atomizer (in which case spores would lodge both on the upper and lower surfaces of the leaves) good infection was obtained, whereas when the inoculum was placed only on the upper leaf surface, there was either no infection at all, or only a very slight amount of it. This led to a rather detailed investigation of the mode of infection of these two serious parasites of the strawberry, the methods and results of which form the substance of this paper.

HISTORICAL

The literature bearing directly on this subject is rather limited. Most of the papers dealing with the strawberry leaf blights either make no mention of the mode of infection, or simply state that the spores fall on the leaves, germinate, and enter the leaf tissue, without giving any definite information as to whether infection takes place through the upper or the under leaf surfaces and whether the ingress is through the stomata or by penetration of the epidermis. Garman (4) states: "As these latter (spores) ripen, they are set free, are scattered upon fresh leaves by winds and rains, to germinate there, push into the leaves, and form new spots." The use of the term "push" would perhaps suggest that Garman considered ingress to be by penetration of the epidermis rather than stomatal. Two papers, Dudley's (3) on leaf spot, and Wolfe's (8) on scorch, have a direct bearing on the mode of infection, and these are reviewed somewhat in length as the findings of these workers are, for the most part, at variance with those of the writer.

Dudley (3) claims infection with Mycosphaerella may take place through both the upper and the lower leaf surfaces and that the germ tube enters not through the stomata but by *boring* through the epidermis. He

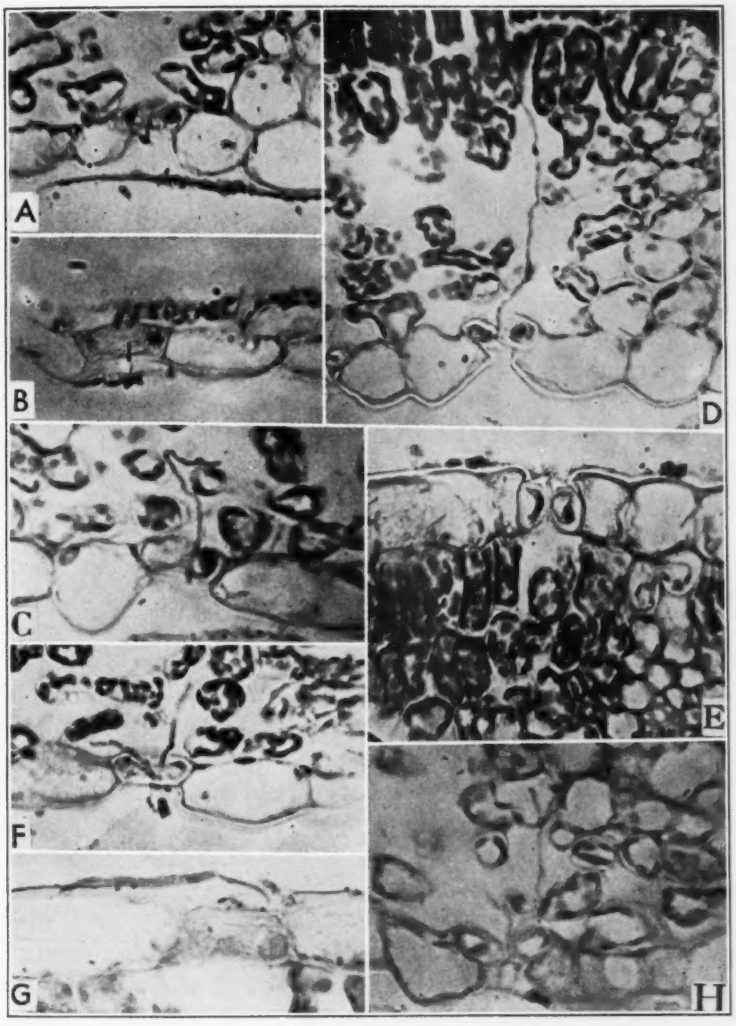

FIG. 1. Mode of infection in *Mycosphaerella fragariae*. A and B. Very early stages of infection. Young germ tubes just entering stomata of the lower epidermis. Three days after inoculation. ×386. C. Germ tube has entered through a stoma and is curving around a mesophyll cell. Five days after inoculation. ×500. D. Germ tube has entered through stoma of under epidermis and is growing upward toward the palisade cells and upper epidermis. Four days after inoculation. ×386. E. Typical stoma on the upper epidermis of the Klondike variety of strawberry. ×386. F and H. Lower epidermal stomata with 2 germ tubes (or branches of the same germ tube ?) entering. Seven days after inoculation. ×500. G. Germinating spore on the upper epidermis, its germ tube curving toward an upper epidermal stoma. ×500.

states (p. 175): "Numerous infections were made in April and May, by placing both germinated and ungerminated conidia on the upper and the under surfaces of young strawberry leaves, which we believed to be previously uncontaminated by the fungus. It was found that the germ tubes bored their way between the epidermal cells of the upper surface, but they were not observed to enter by the stomates of the under surface, although infection took place readily from that surface. Entrance by the stomates was certainly not the usual mode of attack." No photographs or other illustrations of germ-tube penetration are shown, and the variety or varieties experimented with is not designated. This latter point is important, for in the present investigations it was found that the leaves of some strawberry varieties have stomata on both surfaces.

The writer's findings are not in accord with those of Dudley. Germ-tube ingress was repeatedly found to be stomatal (Fig. 3); if ingress can occur also by penetration of the epidermis, it was never observed during the course of these studies.

Wolf (8), in describing the development of acervuli and conidia of both the strawberry fungus, *Diplocarpon earliana*, and the rose fungus *D. rosae*, states (p. 10): "The essential features in the development of the conidial stage of the strawberry fungus and the rose fungus are entirely similar. In the case of each, infection occurs by direct penetration of the germ tubes from conidia which lodge and germinate on the upper leaf surface. Within a few days this hypha will have developed a subcuticular mycelium. Meanwhile certain branches will have extended downward between the epidermal cells and between the subjacent palisade parenchyma and mesophyll." Dodge (2) also reports the formation of a subcuticular layer of mycelium in the case of the rose fungus.

The writer did not include the rose fungus in his studies, but his findings on the mode of infection of the strawberry fungus do not agree throughout with those of Wolf. He agrees with Wolf that ingress occurs by direct penetration of the epidermis by the germ tubes, but finds that infection takes place primarily, if not wholly, through the lower, rather than the upper, leaf surface. Furthermore, no subcuticular mycelium was ever found to develop from the germ tube. The only subcuticular mycelium observed was the stroma from which the young acervulus arises and that makes its appearance at a later stage, just prior to the beginning of the young acervulus.

MATERIALS AND METHODS

The Klondike variety of strawberry was used in all the infection experiments with Diplocarpon and in all but two with Mycosphaerella. Potted plants were used in all cases. The inoculated plants were kept either in the laboratory by the window, in the greenhouse, or outdoors.

The inoculum consisted of conidial suspensions in water made from pure cultures of the respective organisms grown either on bean-pod agar in plates or on bean pods in tubes. The inoculum was smeared on the upper or lower leaf surface by means of a camel-hair brush. When the material was intended for histological studies, the inoculum was smeared on definite leaf areas marked with India ink. The inoculated plants were kept under moist bell jars for 2 to 3 days after inoculation.

The material for paraffin imbedding was fixed at different intervals from the 2nd to the 7th day after inoculation. Gilson's and the alcohol-acetic acid-formalin (100 cc. of 50 per cent alcohol, 6.5 cc. of formalin, and 2.5 cc. of glacial acetic acid) fixatives were used. The latter was found more satisfactory. Various stains were tried, including Pianeze III B (7), Dickson's (1), Ridgway's (5), Flemming's triple, Haidenhain's haematoxylin, and Stoughton's (6) thionin-orange G. Both the haematoxylin and the thionin stains proved satisfactory, but the latter was used most extensively because of the easiness and the shortness of the process.

EXPERIMENTAL

The experimental work was carried on simultaneously with both fungi but, for the sake of clearness, the results obtained with the two organisms are presented separately.

INFECTION EXPERIMENTS

I. *Diplocarpon earliana.*—The inoculation experiments with *D. earliana* and the results obtained are, for convenience, summarized in table 1. The lower-surface inoculations, in all except the first experiment, resulted in very heavy infections. Hundreds of spots developed in every case on every leaflet inoculated on the lower surface in 10 to 14 days. These spots would coalesce and "scorch" the leaves, often killing them completely in 18 to 21 days. Acervuli and conidia developed in abundance, and always on the upper surface. Dodge (2) found that the acervuli of *D. rosae* occasionally occur on the lower leaf surface, and he considers this as an indication that infection sometimes may occur naturally through that surface. In these experiments where infection was known to have taken place through the lower surface, the acervuli were found to develop only on the upper surface.

The relatively light infection in experiment I (Table 1) is thought to be due to the fact that the inoculated plants were not placed under moist bell jars, and there was not sufficient moisture for the spores to germinate.

The upper-surface inoculations resulted either in no infection at all (Experiments I, III, IV, V and VII, Table 1), or in very light infection (Experiments II and VI, Table 1). It is not certain whether the infection in

TABLE 1.—*Results of inoculations with Diplocarpon earliana on the upper and under surfaces of the leaves*

Experiment number	Date	Location	Number plants inoculated		Results	
			Upper	Under	Upper	Under
I[a]	2/15/29	Greenhouse	3	3	No infection	4 spots on one plant, 10 on another, and no infection on third
II	3/11/29	Greenhouse	3	3	Very light infection, only occasional spot here and there (Fig. 2, A and B)	Hundreds of spots on every inoculated leaf (Fig. 2, A and B)
III	11/19/29	Laboratory	1	1	No infection	Hundreds of spots on every inoculated leaf
IV[b]	11/27/29	Greenhouse	2	2	No infection in 3 weeks	Minute spots appeared in 12 days. By end of 3 weeks, hundreds of coalescing spots "scorched" the leaflets
V	12/4/29	Laboratory	1	1	No infection	Hundreds of coalescing spots on every leaf
Check[c]	12/4/29	Laboratory	1	1	No infection	No infection
VI	1/9/30	Greenhouse	2	2	8 spots on one plant and 5 on the other	Hundreds of spots on every leaf
Check[c]	1/9/30	Greenhouse	1	1	No infection	No infection
VII[d]	2/4/31	Laboratory	4 (18 leaflets)	4 (16 leaflets)	No infection	Hundreds of coalescing spots on each leaflet

[a] Inoculated plants not placed under bell jars.
[b] Plants sprayed with sterile water and placed under bell jars 7 hours before inoculation.
[c] Surfaces brushed with sterile water.
[d] The same 4 plants were used for both upper and under surface inoculations, some of the leaflets on each plant being inoculated on the upper and some on the under surfaces and some being left uninoculated as checks.

these two experiments actually occurred through the upper surface, or whether it was due to accidental contamination of the lower surface. It may or may not be significant that when the inoculated plants were kept in the laboratory (Experiments III, V, and VII, Table 1) and extreme caution was used in the process of inoculations (all the overlapping leaflets were removed so there would be no touching between the upper and lower surfaces of the inoculated leaflets) no infection ever resulted from the upper-surface inoculations.

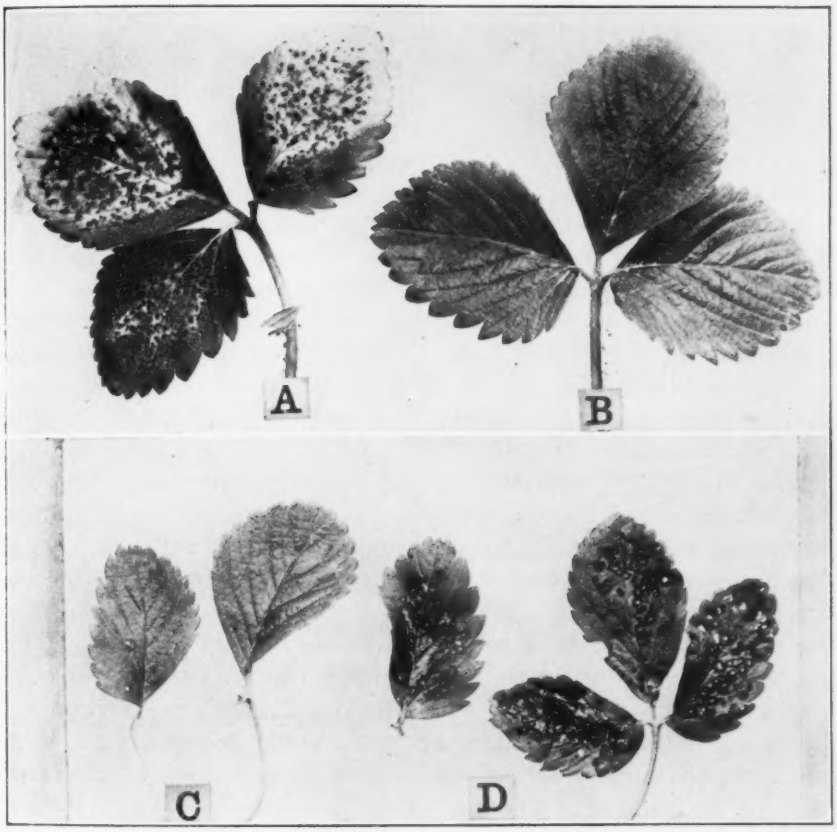

FIG. 2. A and B. Results of inoculating leaves with *Diplocarpon earliana*. A. Lower surface. B. Upper surface. C and D. Results of inoculating leaves with *Mycosphaerella fragariae*. C. Upper surface. D. Lower surface.

Since the inoculation was done by smearing the inoculum onto the surface by means of a camel-hair brush and consequently some rubbing of the surface and breaking of trichomes occurred, it was feared that some of the spots might be due to mechanical injury of the epidermis rather than

points of fungal infection. To test this possibility, check inoculations were included in some of the experiments (Experiments V and VI, Table 1) in which the brush was dipped in sterile water and rubbed on the leaf surfaces in the same manner as when inoculum was used. No spots developed and no injury to the surface, either to the upper or to the under, was noted in these cases.

HISTOLOGICAL STUDIES

Several methods were tried in the effort to determine the mode of infection. The Pianeze III B differential stain (7) was first tried. Pieces of the inoculated leaves were fixed in a mixture of equal parts of 95 per cent alcohol and acetic acid. After the chlorophyll was removed by several changes of 70 per cent alcohol, the bleached leaf pieces were stained, mounted, and examined. This method proved unsatisfactory, for the thick covering of trichomes on the under surface interfered with the field of vision.

Attempts were made to strip the epidermis of inoculated leaves and examine it to see how the germ tube enters. The strawberry leaf epidermis does not strip readily, however, and only very small pieces could be examined. Since the scorch fungus attacks, besides the leaf blades, also the petioles and the fruit-cluster stems, and since the epidermis of the latter 2 organs strips very readily, the method was modified and petioles and fruit stems were used. Beginning the second day after inoculation, pieces of the epidermis were stripped off, mounted in a 10 per cent solution of glycerine in S. T. 37 (Hexylresorcinol), stained on the slide with a very dilute solution of eosin, and examined. The fungus mycelium stains much more deeply than the epidermal cells and can be seen very easily. This method proved only partly successful. It did not show how the germ tubes enter, but it gave a strong indication that ingress was not stomatal. Hundreds of stomata were examined, but in no case was a germ tube found entering through a stoma. On the other hand, germ tubes often were observed growing over a stoma, or very near it, without entering.

The method finally adopted was to make paraffin sections of the inoculated leaves. While this method was long and time-consuming, as hundreds of slides were made and thousands of sections examined, the satisfactory results obtained justified its use. The material was killed and fixed at one-day intervals, beginning 24 hours after inoculation and continuing through the seventh day. Leaf material from natural scorch infection in the field also was included. This material represented the earliest visible stages of infection, very young spots, barely visible to the naked eye, and spots slightly older, of pin-point to pin-head size. The sections were cut from 6–12 μ thick and both parallel and vertical to the surface.

Findings: Over 2,000 sections of leaf material, fixed from the second to the seventh day after inoculation, were examined. In no case was the fungus found entering from the upper surface. It is not claimed that infection cannot take place through the upper surface; in fact, the results of some of the inoculations (Experiments II and VI, Table 1) might indicate that occasionally infection does occur through that surface. Under the conditions of these experiments, however, fungus ingress through the upper surface either did not occur, or was so rare that its ocurrence was not observed in the examination of the sections. On the other hand, entrance through the lower surface frequently was observed (Fig. 3 and Fig. 4, C).

The germ tube is rather thick and of irregular diameter (Fig. 4, A and B) and stains somewhat lighter than the young branch hyphae arising from it after penetration. It enters the tissue by penetrating between the epidermal cells (Fig. 3 and Fig 4, C), and grows directly to the mesophyll

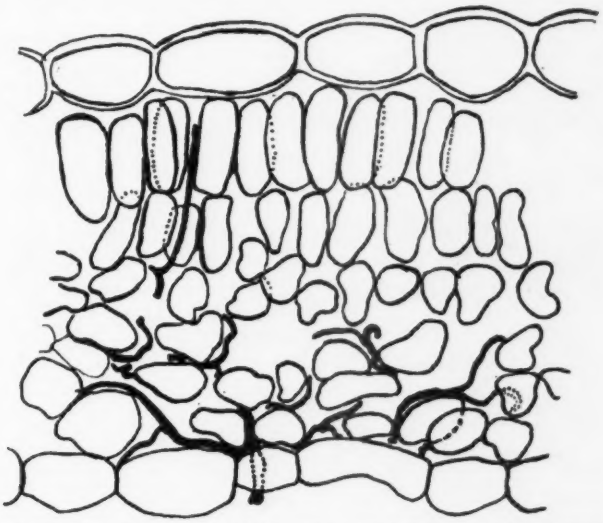

FIG. 3. Mode of infection with *Diplocarpon earliana.* Point of fungus ingress and relation of mycelium to host tissue are shown. Drawn from a photomicrograph made 5 days after inoculation. ×386.

layer without forming any subcuticular layer of mycelium. No subcuticular mycelium was ever observed, either on the upper or the lower epidermis, either in sections of relatively young material (1 to 5 days after inoculation) or in sections of young spots (age of infection unknown) on naturally infected leaves collected in the field. It never was seen to enter by penetrating the epidermal cells, themselves, nor through the stomata. On the contrary, germinating spores often were observed in the immediate vicinity of stomata, sometimes lodged directly over them, the germ tubes

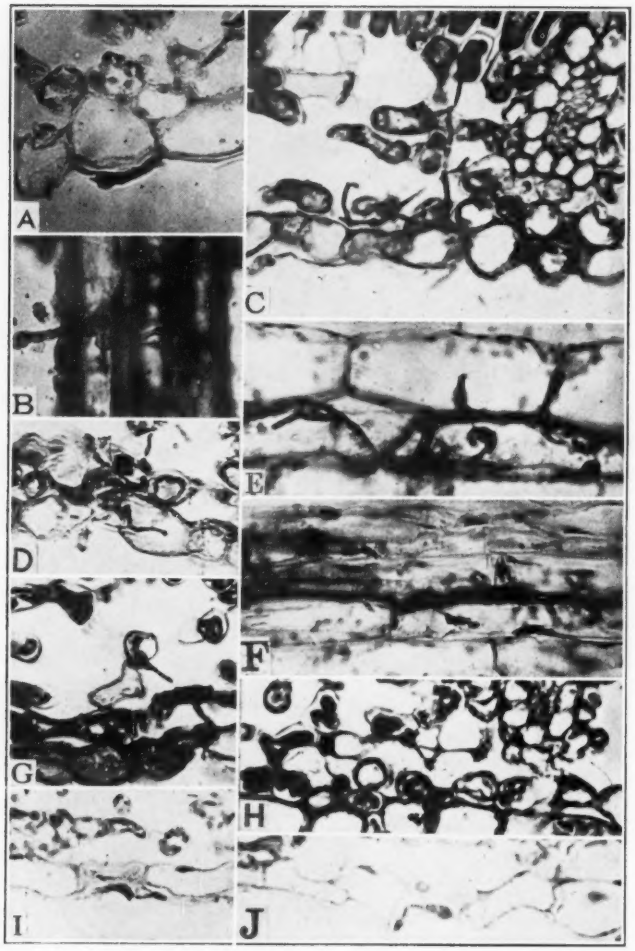

FIG. 4. Mode of infection in *Diplocarpon earliana*. A. Germ tube following the contour of a lower epidermal cell, its point pressing against the middle lamella. Material killed and fixed 3 days after inoculation. ×400. B. Longitudinal section of fruit-cluster stem showing penetration by germ tube. Material killed and fixed 5 days after inoculation. ×400. C. Cross section of leaf, showing mycelium in the tissue and point of entrance between 2 lower epidermal cells. The outside remnant of the germ tube is seen (slightly out of focus) extending from the point of entrance outward. Material killed and fixed 3 days after inoculation. ×329. D. Haustorium (?) in epidermal cell. ×320. E and F. Longitudinal sections of fruit-cluster stem showing haustoria in cells. Material killed and fixed 5 days after inoculation. E, ×432; F, ×320. G. Club-shape hypha (haustorium mother cell?) pressing against a mesophyll cell that shows injury. H. Hypha similar to G, its thickened end pressing against a mesophyll cell and its pointed end inside another mesophyll cell. Both cells have collapsed. Material fixed 3 days after inoculation. ×320. I and J. Germinating spores lodged over stomata, their germ tubes growing away from the stomatal openings.

growing away from, rather than toward, the stomatal opening (Fig. 4, I and J). Once inside the tissue, the germ tube branches rather freely, and gives rise to hyphae that grow between the mesophyll cells (Figs. 3 and 4, C). In the early stages, the mycelium is confined to the mesophyll layer. Later, branch hyphae grow upward toward the palisade cells and the upper epidermis (Figs. 3 and 4, C). In the beginning, the cells in the vicinity of the mycelium do not seem to be injured, judging from their appearance and staining reactions. When injury becomes apparent (collapsed or discolored cells), it is first manifested in the lower epidermal and mesophyll cells.

In the leaf tissue haustoria are not apparently abundant, at least in the early stages of infection. In all the leaf material examined, only 3 cases of structures resembling haustoria were observed. One is a hypha within an epidermal cell of the lower surface (Fig. 4, D). This may be considered as either an intracellular hypha or as a haustorium. Since the mycelium in the tissue was found to be intercellular, this structure is assumed to be a true haustorium, even though, in form, it is not typical of the definite haustoria found in the cells of the fruit stems (Fig. 4, E and F). The other 2 structures were observed in the mesophyll tissue of material killed 3 days after inoculation. One (Fig. 4, G) is a club-shape hypha with its thickened end pressing against a mesophyll cell. The cell is not collapsed, but apparently is injured, as shown by its staining reaction. This club-shape hypha is assumed to be a haustorium "mother cell." A similar hypha is seen in figure 4, H. Its thickened end is pressing against a mesophyll cell and its opposite end has penetrated a second cell. Both cells have collapsed and stain brown instead of deep purple, like the healthy cells.

In the tissue of the peduncles, on the other hand, haustoria were found in abundance (Fig. 4, E and F). These are very similar to those of *Diplocarpon rosae,* described by Dodge (2). They may be rather long and hypha-like, short and thickened, of irregular diameter, straight or curved, and with or without a sheath at the base (Fig. 4, E and F).

Infection Experiments

II. *Mycosphaerella fragariae.*—As brought out in table 2, in which the results of the inoculation experiments with the leaf-spot fungus are summarized, infection with this fungus also occurs usually through the lower leaf surface. Either there was no infection (Experiments II, III, V, VI, VII, VIII, and X, Table 2), or very light infection, compared to that on the under surface (Experiments I, IV, IX, and XI, Table 2) when the inoculations were made on the upper leaf surface. Infection from inoculations on the lower surface was, on the other hand, readily obtained and in most cases it was extremely abundant, hundreds of spots developing on every inoculated leaflet.

TABLE 2.—*Results of inoculations of the strawberry with Mycosphaerella fragariae on the upper and under surfaces of the leaves*

Experiment number	Date	Location	No. plants inoculated		Results	
			Upper	Under	Upper-surface inoculation	Under-surface inoculation
I	2/15/29	Greenhouse	3	3	One spot on each of 2 plants; no infection on the third	10 spots on one plant, 36 on another, and 21 on the third
II	3/11/29	Greenhouse	3	3	No infection	3 spots on one plant, 10 on the second and no infection on third
III	11/19/29	Laboratory	1	1	No infection	15 spots
IV[a]	11/27/29	Greenhouse	3	2	8 spots on one plant, 2 on another, and 12 on the third	35 spots on one plant and over 100 on the second
V	1/9/30	Greenhouse	2	2	No infection	No infection
Check[b]	1/9/30	Greenhouse	2	2	No infection	No infection
VI	2/6/30	Greenhouse	3	3	No infection	Hundreds of spots on one plant; no infection on the other two
		Outdoors	2	2	No infection	16 spots on one plant; no infection on the other
VII[c]	3/3/30	Greenhouse	2	2	No infection in 20 days	Hundreds of spots on all the older leaves in 4 days. No infection on younger leaves in 20 days
		Outdoors	2	1	No infection in 20 days	Hundreds of spots on all older leaves in 4 days. No infection on younger leaves in 20 days
VIII[d]	4/19/30	Greenhouse	3	2	No infection	Hundreds of spots in 4 days
		Greenhouse	2	1	No infection	Hundreds of spots in 4 days
IX[e]	11/19/30	Laboratory	1	1	Six spots	Hundreds of spots, coalescing and killing the leaves
X[e]	1/7/31	Laboratory	2 leaves (same plant)	2 leaves	No infection	Very numerous spots
XI	12/4/31	Outdoors	4	4	Spots only around insect holes in leaves (Fig. 2, C and D)	Spots very numerous on all inoculated leaves. (Fig. 2, C and D)

[a] Plants sprayed with water and placed under bell jars 7 hours before inoculating.
[b] Sterile water.
[c] The plants used in this test were those that showed no infection in experiment VI. (See explanation in text, p. 623.)
[d] Same plants as those used in experiment VII. All the leaves showing infection were removed and the plants were placed under moist bell jars without reinoculation.
[e] Seedlings of the variety "Banner."

It is considered necessary to explain the abnormal results of experiments VII and VIII (Table 2), in which spots became visible in the short period of 4 days. In experiment VII, the plants employed were those that showed no infection in experiment VI (3 greenhouse and 2 outdoor upper-surface-inoculated and 2 greenhouse and 1 outdoor lower-surface-inoculated plants). Since these plants showed no signs of infection in 25 days, the inoculations were considered ineffectual. Accordingly, the plants were reinoculated, as shown in table 2, on March 3, 1930, and placed under moist bell jars for 3 days. On the fourth day, very numerous spots appeared on the older leaves of the plants inoculated on the lower surface. These spots increased in size during the next few days and eventually became typical of this disease. Four days is too short a period for infection to take place and become externally manifest (the incubation period for strawberry leaf spot is usually 10 to 14 days). The most probable explanation, in the writer's opinion, is that infection had taken place from the first inoculation (February 6) and that the fungus lay in the tissue in a more or less dormant state and resumed growth and became externally manifest when conditions (high humidity) for it became favorable. Additional evidence in support of this explanation is the fact that the spots appeared in 4 days only on the outer leaves, those that were included in both the first and the second inoculations, but not on the young leaves, which were not included in the first inoculation. To test this supposition further, the same plants were used in experiment VIII. In this experiment, all the outer leaves (those included in both inoculations) and all of the younger ones that showed any signs of infection were removed, and the plants, without being reinoculated, were placed under moist bell jars for 3 days. Again, on the fourth day, very numerous spots appeared on all the leaves that had been previously inoculated on the lower surface. No infection occurred on those that had been inoculated on the upper surface.

Dudley (3) reports a somewhat analogous case of dormancy of the Mycosphaerella fungus in the tissue. He states (p. 175): "In the early spring, it was found that wholly new spots would appear on diseased leaves, in so short a time after they were brought into the laboratory that no infection could have taken place from newly formed conidia. Probably these diseased areas arose from a fresh growth of mycelium that had hibernated in the leaf."

HISTOLOGICAL STUDIES

In general, the same methods described for Diplocarpon were followed in investigating the mode of infection of the leaf-spot fungus. Ingress in the case of Mycosphaerella was found to be wholly stomatal (Fig. 1). In all the material examined, no indication that the fungus enters by penetration of either the upper or the lower epidermis was ever observed. If this

[c] The plants used in this test were those that showed no infection in experiment VI. (See explanation in text, p. 623.)
[d] Same plants as those used in experiment VII. All the leaves showing infection were removed and the plants were placed under moist bell jars without reinoculation.
[e] Seedlings of the variety "Banner."

mode of ingress did occur, it was not found during the progress of these investigations. On the other hand, entrance through the stomata was observed very frequently (Fig. 1, A, B, C, D, F, and H). The germ tube is of about the same thickness as the mycelium of this fungus and in the early stages stains very deeply. It grows and ultimately reaches a stoma. After entering the stoma it may curve and continue its growth between the mesophyll cells, (Fig. 1, C and F), or, often, it may grow straight upward between the palisade cells (Fig. 1, D) toward the upper epidermis. No haustoria or intracellular mycelia were found.

Assuming that the stomata of the strawberry occur only on the under epidermis, it was puzzling at first to reconcile the occasional upper-surface infection (Table 2) with the observed fact that ingress is stomatal. It was soon observed, however, that in the Klondike variety, at least, stomata are not confined to the lower leaf surface alone; they are present also on the upper surface (Fig. 1, E and G), although in much smaller number (Table 3) than on the lower surface. While no actual entrance through an upper-epidermis stoma was observed, germinating spores were often seen in the vicinity of such stomata, and it is believed that the occasional infection obtained from upper-surface inoculations occurred through stomata. Figure 1, G, shows a germinating spore next to an upper-surface stoma, the germ tube curving toward the stomatal opening.

STOMATA AND VARIETAL RESISTANCE

Having determined that infection with the leaf-spot fungus is stomatal and that the variety studied (Klondike) possessed stomata on both leaf surfaces, the thought of determining whether or not there was a correlation between number of stomata and varietal susceptibility to this disease suggested itself. In June, 1931, while visiting the strawberry-breeding farm at the United States Plant Field Station, Glenn Dale, Maryland, the writer had opportunity to collect leaf material from resistant and susceptible varieties for study. In this variety plot, where leaf spot was abundant, some varieties were found very severely spotted, while others, growing alongside diseased ones, were disease-free. The latter were considered resistant. The material was collected and placed in killing solution on the spot and later imbedded in paraffin. Some of this material, unfortunately, was lost when the temperature control of the box in which the material was kept got out of order and the paraffin blocks melted, but new material, partly replacing that that was lost, was received through the kindness of G. F. Darrow in October, 1932.

Table 3 summarizes the findings. The figures may not be absolute, and are probably high, because the stomata were counted on cross sections of the leaf, cut 8–10μ thick; and so, if a stoma were cut in two, it would

TABLE 3.—*Comparison of the average number of stomata per square millimeter of leaf surface of strawberry varieties resistant or susceptible to Mycosphaerella fragariae*

Variety	Type	Total leaf area examined[a]	Average number of stomata per sq. mm.	
			Upper	Under
Klondike	Slightly resistant	36.81 sq. mm.	5.54	265.6
Howard 17 × 633	Resistant	51.14	0	237.7 +
Missionary × ?	Very susceptible	39.40	0	302.5
Klondike × 817	Susceptible	14.58	0	233.6 +
N. Y. Station No. 4626	Very susceptible	20.80	0	287.0
Howard[17]	Very resistant	32.14	0[b]	304.7

[a] Sum of many samples taken from several different leaves.
[b] Only one stoma found on entire 32.14-mm. area examined.

appear on 2 successive sections and would be counted twice. However, since the same method was used in all cases, the figures are satisfactory for comparison. The results obtained do not indicate that there is a direct correlation between the number of stomata in a variety and resistance or susceptibility to leaf spot. Actually, there was more variation found between different leaves of one variety, or even between portions of the same leaf, than between the average figures of resistant and susceptible varieties.

SUMMARY

Inoculation tests with *Diplocarpon earliana* (Ell. & Ev.) Wolf and *Mycosphaerella fragariae* (Tul.) Lindau showed that infection with both organisms takes place, predominantly, if not wholly, through the lower leaf surface. The variety Klondike was mainly used in these tests.

Histological studies showed that in the case of Diplocarpon, the germ tube enters by direct penetration between the epidermal cells. It was never observed to enter through the stomata. No subcuticular mycelium was ever observed. After the germ tube reaches the mesophyll layer, it branches and the intercellular mycelium grows between the mesophyll cells for some time before branch hyphae reach the palisade cells and the upper epidermis. Haustoria were found. These were more abundant in the pedunele than in the leaf cells.

In the case of Mycosphaerella, ingress takes place through the stomata. In no case was it ever found to be other than stomatal. The Klondike variety was found to have stomata on the upper epidermis, also, but they

were decidedly fewer than on the lower surface.

No correlation was found between the number of stomata and susceptibility or resistance to leaf-spot infection in 6 varieties studied.

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THE TRAP CROP AS A MEANS OF REDUCING ROOT-KNOT-NEMATODE INFESTATION[1]

G. H. Godfrey and Helene Morita Hoshino

(Accepted for publication August 26, 1933)

The use of the trap crop as a means of contributing to the control of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey[2] has never, in recent years, received serious consideration. Theoretically, the method is sound. In connection with a long series of experiments conducted by the nematology department of the Pineapple Experiment Station in Hawaii, designed to test every known method of nematode control, several experiments on the trap-crop method were carried through. An account of one series, in cans and tubs of soil, is included in this paper. Another series, conducted under conditions more nearly approximating those of the pineapple fields of Oahu, is reported in another paper (13).

LITERATURE REVIEW

The trap-crop (Fangpflanzen) idea as applied to the control of the plant-parasitic nematodes originated with Kühn who, as early as 1881 (22), experimented with it as a means of reducing soil infestations of *Heterodera schachtii* in sugar beets in Germany. He continued to work with it up to 1886, when he reported at length (23) on a series of several years' experiments. He demonstrated a high degree of efficiency in control with summer rape, *Brassica rapa* L., as a trap crop, and was able to show marked improvement in sugar-beet growth and yield following his treatments. In a much later paper (24) his enthusiasm for the method as a practicable one for field application still holds, and in 1909, according to Müller and Molz (28), he presents a scholarly review of the method and its possibilities.

Several other European students of the sugar-beet-nematode problem make favorable mention of the trap-crop idea without adding materially to it. Among these are Ritzema Bos (30), Fream (9), Marcinowski (25), Blunck (4), and several others, references to whose papers and to the progress reports of Kühn through several years are to be found in the valuable bibliography on the genus Heterodera, issuing from the Imperial Bureau of Agricultural Parasitology in England (20). Spiegler (32) states that he has obtained marked results from it in reduced nematodes and

[1] Published with the approval of the Director as Technical Paper No. 60 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] According to the international code of zoological nomenclature this is the correct name for the root-knot nematode, which has long been erroneously called *Heterodera radicicola*. See papers by Goodey (18) and Tyler (34).

increased sugar-beet yields, but that he has abandoned the method because of the danger, in field practice, of actually increasing nematodes rather than reducing them and because of the expense involved in the treatment. Müller and Molz (28) contribute to the trap-crop idea the thought of destroying trap crops at the right stage of growth by means of a poison spray rather than by mechanical means. They present convincing data of decrease in nematode population and increase in crop yield. Molz (27) later practically dismisses the trap-crop method from practical consideration, along with other methods of direct control, with the statement "Praktisch wertlos war das Kühnsch Fangpflanzenverfahren . . . ," and refers with greater hopefulness to the method of stimulating the hatching of eggs with various stimulating substances, developed in part by himself (26) and by Baunacke, Rensch, and Nebel, to whom he refers. Various of these investigators, who worked mostly in Germany, mention the facts of failure to kill larvae that have recently entered roots, and the continued development of some of those that entered earlier to the egg-producing stage if the plants were killed only slightly too late.

There is no record available to us of any investigations in America on the trap-crop method for sugar-beet-nematode control. Shaw (31) reviews the work of the European investigators up to 1915, quoting Spiegler (32) in particular. He appears inclined to agree with Spiegler and discards the trap crop from further consideration for the control of the sugar-beet nematode in America. Other American contributors on the control of this nematode follow Shaw in offering no encouragement for the trap-crop idea.

The early developmental stages of the root-knot nematode, *H. marioni* are very similar to those of *Heterodera schachtii*. Both penetrate completely to the interior of young roots and become established in the tissues where they remain fixed until maturity. No doubt, because of these similarities, there is considerable mention in the literature of the trap-crop method as a possible means of control of the gall-forming nematode. Frank (8) lists it as one of the possible means of control, without experimental support. Cobb (5, 6) in Australia quotes Kühn's method in detail and considers it favorably as a control measure for the root-knot nematode but reports no experimental work on it. Atkinson (1) mentions the trap-crop method of control. Stone and Smith (33) list it along with other methods of control, quoting the European investigators Kühn and Hollrung as authorities. They contribute no original work on the method. Bessey (2) cites the European investigations and considers their possible application for root knot nematode control. He conducted experiments in the southern States in America for 2 years with varying results. Since treated plots and check plots did not show noticeable differences in succeeding crops, some of the plants remaining free, some slightly affected, and some very badly affected by root

knot, Bessey decides adversely as to the value of the trap-crop method. He and Byars (3) do not include the method in their list of recommendations for nematode control in their *Farmers' Bulletin* on the subject, nor does Godfrey (10) in his. Godfrey (11, p. 246) reports a great reduction in nematode content of soils used a second time, after a first test in which plants were removed before the propagation of a new generation of nematodes. Godfrey and Morita (14) reported briefly on a phase of the studies included in the present paper, in an Academy of Science Proceedings not widely distributed. This paper is cited because of its listing in the general Heterodera bibliography (20) previously mentioned. The present paper gives a much more complete report. No other investigator of the root-knot problem in America contributes any new information on the trap-crop method.

BASIC CONSIDERATIONS

The theory of the use of the trap crop for trapping this nematode from the soil is based on the fact that, once established inside the root, the larvae of *H. marioni* are incapable of further motility. Not until the males are fully mature do they make their final moult from the fusiform body wall and migrate to the exterior or to another position within the root. The female does not move away from her fixed position at all. This is fully elucidated in an earlier paper from this Station (16), in one by Nagakura (29) in Japan, and elsewhere. The original larvae, then, are virtually imprisoned within the roots and are removed from all possibility of parasitizing another plant. It is only their progeny that can bring about new infection. The trap-crop idea, then is simply to plant a highly susceptible crop in the soil and to "catch" as many nematodes as possible in the root system. Before the stage of development is attained at which a new generation will develop, the plants are plowed under or otherwise killed and the roots die, resulting in the death of the contained nematodes. The roots need not necessarily even be removed from the soil so long as all possibility of continued growth is eliminated. The rate of reproduction of this nematode is so rapid that only a few new colonies, started by the development and hatching of egg masses, would quickly overcome the benefit derived from the trapping of the original population. The enclosed immature females must not have attained that critical stage where they will continue to develop and produce eggs, even if the host plant roots are not functioning. The element of time of destruction of the host plant, therefore, is a very important one in connection with the use of the trap crop.

There are peculiar difficulties incident to studies on soil populations of this nematode that have thus far made it impossible, by any known method of taking a soil census, to account for all the nematodes present in a soil

at any particular time. Another paper (12) deals at length with this subject. In the experiments herein recorded this fact becomes particularly evident. Differences in soil type, soil moisture, season, condition of the nematodes, natural enemies, host-plant growth, and other factors controlled with difficulty, make for lack of uniformity in results in different lots of soil receiving supposedly identical treatments. For any one experiment these factors are made as uniform as possible except for the controlled variables in all lots. Different experiments, however, are probably not strictly comparable.

EXPERIMENTS

The experimental work reported in this paper comprises 4 experiments conducted from 1926 to 1932.

A Test of Period of Growth in Relation to Trapping, with the Tomato as a Trap Crop

Experiment 1. This experiment, started in August, 1926, had 2 objectives, the first to determine the length of life cycle of the root-knot nematode under Hawaiian conditions, and second, to determine the practicability of the trap-crop idea. Tomato plants were grown in flats of sterilized soil and later transplanted to small pots to promote good root growth. In the meantime soil heavily infested with nematodes was thoroughly mixed and placed in a series of about 30 1-gal. cans. When the tomato plants were well established they were transplanted, without disturbing the root systems, into the centers of the gallon cans, and growing conditions were maintained. The tomato plants were in such condition that rapid development of good root systems took place. According to plan, 3 plants were removed on every third day, the roots washed thoroughly to free them from soil, and examined and records were made of the number of galls and of stage of development, according to the indicator-plant method reported in another paper (12). Following this 2 separate procedures were followed. (1) A second planting of tomatoes was made in the same soils from which the first 3 plants had been removed. This second planting remained in the soil in every case only until just before the egg producing stage of the parasite, as indicated by observations on separate plantings. At this time they were removed and still another planting made. This was continued until galls were no longer found. In this way, no reproduction occurring in the soil, each successive planting reduced the nematode count of the soil more and more. The gall count indicated rate of reduction. (2) The roots removed from the soil in every case were carefully washed to remove all possibility of surface infection and were used to inoculate pots of sterilized soil. Into these pots tomato plants previously started in sterile soil were planted. By this means it was possible to determine to what extent nematodes capable of causing new infection were carried over inside the roots.

The results in numbers of knots on the roots of tomato plants in 4 successive plantings in the same soils, are indicated in table 1. The roots of the tomato plants in lot 1, a, b and c, showed no galls when removed 3 days after inoculation. Obviously this was too short a period for the larvae to find the roots or, if infection did occur, for galls to have become evident. The nematodes left in the soil infected the second plant. This explains the greater abundance of root galls in the second planting in this case. When

TABLE 1.—*The effect on subsequent infection of periodic trapping by removal of infected roots from soil badly infested with Heterodera marioni*

Lot number	1st planting			Subsequent plantings (all 24 days)					
				2nd planting		3rd planting		4th planting	
(All inoculated 9/17/26)	Roots removed after (days)	No. of galls	Av.	No. of galls	Av.	No. of galls	Av.	No. of galls	Av.
1 a	3	0		6		37		0	
b	3	0		103		14		0	
c	3	0	0	44	51	15	22	0	0
2 a	6	23		3		12		0	
b	6	85		35		8		0	
c	6	29	46	2	13	3	8	0	0
3 a	9	203		0		86		0	
b	9	111		0		0		16	
c	9	298	204	3	1	4	30	0	5
4 a	12	493		42		9		0	
b	12	503		0		57		0	
c	12	587	528	0	14	3	23	0	0
5 a	15	1236		10		7		3	
b	15	1011		7		0		0	
c	15	2153	1467	11	9	2	3	0	1
6 a	18	1316		0		81		27	
b	18	2855		0		19		0	
c	18	3903	2691	0	0	8	36	0	9
7 a	21	5000		0		1		0	
b	21	4020		0		2		0	
c	21	2097	3706	0	0	8	4	42	14
8 a	24	3000		13		0		0	
b	24	1200		11		5		45	
c	24	5000	3067	15	13	23	9	3	16
9 a	27	*6092		107		3		0	
10 a	28	**4030		83		13		0	
11 a	29	**6500		56		76		0	
12 a	30	**6500		21		47		3	
13 a	31	**5000		3000		44		2	
14 a	32	**4500		7		57		0	

* Egg masses first found.
** Egg masses abundant.

one considers the low total number of galls for this first lot in the entire 4 plantings, however, it would seem either that high mortality had occurred in the soil, or that a large number of nematodes must have entered the roots without visible symptoms and must have died with the roots when they were removed. The same situation exists for the one or two subsequent lot numbers.

Numbers of galls in the first plantings increased up to the 27th day. This was to have been expected both because of the increased root system with the passage of time, which means simply that more roots are available for infection, and because of the greater prominence of galls. Egg masses were first found on the lot-9 plants, removed 27 days after inoculation. Even here, as well as in the next 3 days, the careful removal of the roots carried most of these egg masses with them, as is shown by the low infections in the second plantings. This shows that in their early development the egg masses adhere very firmly to the roots and likewise that as yet no hatching of eggs has occurred in the soil. At 31 days either the egg masses were easily dislodged or some hatching had already occurred in the soil, for a sudden sharp rise in the amount of infection in the second planting occurs in this lot. The absence of this rise in the lot-14 plants is an irregularity in results that might be accounted for by a soil-texture difference (possibly less water), which permitted the more complete removal of root systems or of a decline in the early hatching of eggs.

In every case after lot 1 there is a decline in gall count in the second planting which becomes more pronounced, proportionately, as the original gall count increases. In most cases this decline is a very abrupt one. It is evident that the majority of nematodes were caught in the first planting. It is likewise evident, however, that not all the nematodes were caught even in the third successive planting, and possibly not even in the fourth. The reasons for this are clear. The original inoculating material consisted in part of undecayed roots containing unhatched eggs and confined larvae which were not released into the soil until after one or more plantings had been removed. That this is an important factor in increasing the longevity of nematodes in the soil is shown by Godfrey, Oliveira, and Gittel (17), Jones (21), and Guba (19). Again, larvae in the soil do not always find their way to roots, even during long periods of exposure of root systems to infection. We have evidence from experiments and observations that the chemotactic movement of larvae through soil to susceptible plant roots is not the all-important factor in infection. Rather, susceptible roots must be growing near where the nematodes are originally located for infection to take place. This point is mentioned again elsewhere in this paper. Müller and Molz (28) show that the same is true for *Heterodera schachtii*.

Figure 1 shows graphically the great difference between the first and

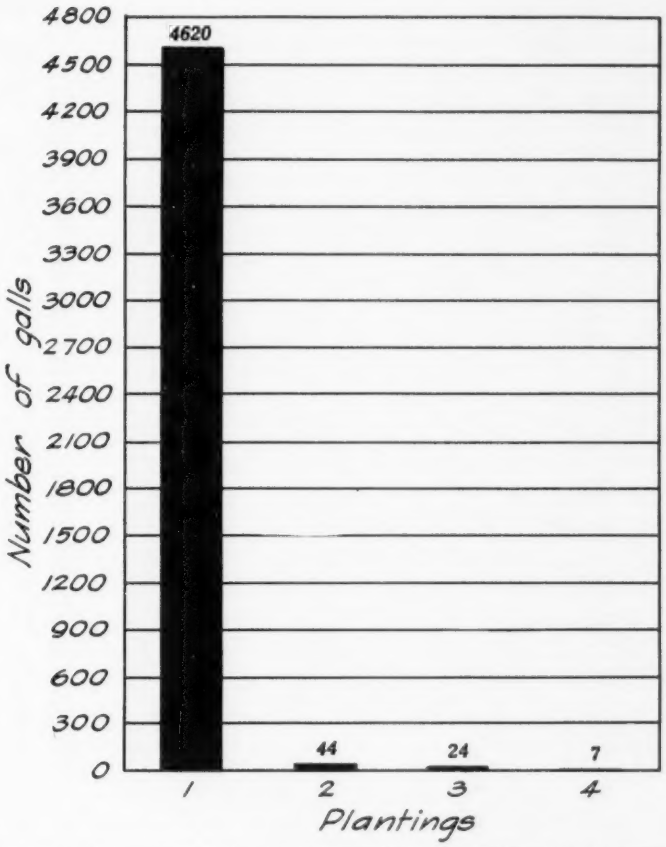

FIG. 1. Graphic representation of the results of experiment 1 in nematode catch, as recorded by count of galls produced, in 4 successive plantings of tomatoes as trap crops. The 4 columns depict the average readings on lots 8 to 12 (see Table 1).

subsequent plantings in number of galls produced on indicator plants up to the time when marked propagation of a new generation of nematodes is evident. This chart is made up of the average gall counts from lots 8 to 12. Over 98 per cent of the total number of galls produced in all 4 plantings occurred in the first. Lot 13, in which increase had occurred, would again show a high reading for the second planting.

Table 2 shows the results of the second phase of the trapping test, made by using the roots removed from the first planting for inoculating new cans of sterilized soil. The inoculum for plant 1 was made up of the entire root systems of plants a, b, and c in lot 1; that for plant 2 of lot 2, a, b, and c, etc. The results are what might have been expected, that is, very low transfer of infection up to the 24th day and then a sudden rise on the 27th day when egg masses were transferred with the roots. The same results are

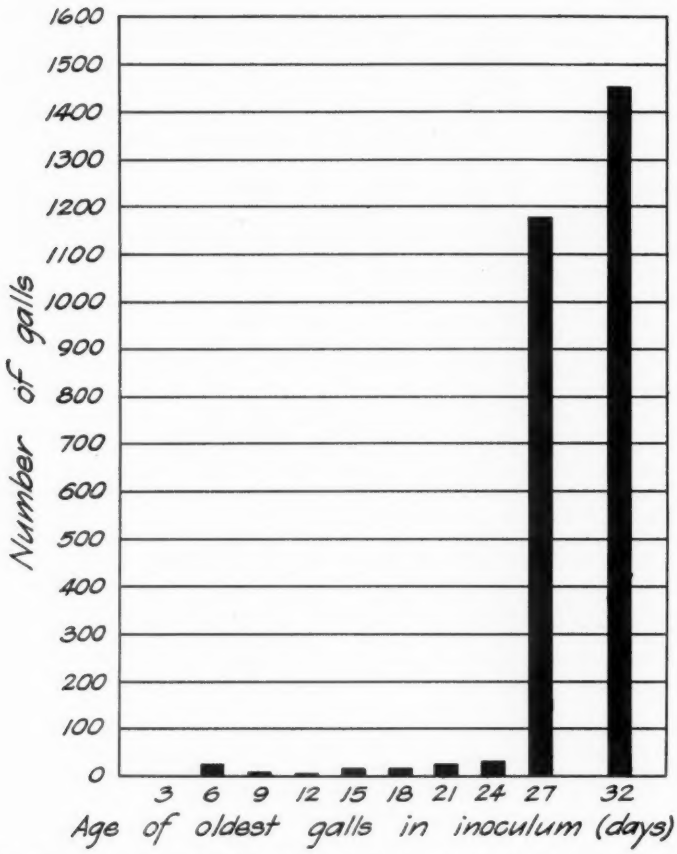

FIG. 2. Graphic representation of the amount of infection in tomato roots grown in soils inoculated with infected roots of different ages. See text for complete explanation and table 2 for exact figures.

depicted graphically in figure 2. The transfer that does occur in the earlier stages was obviously due to very recent infections in the roots in which the larvae, not yet established in the roots, were still capable of moving out into the soil again where they were available for infection of the new plants. The gradual increase in numbers of such infective larvae that were transferred, up to the 24th day, might be expected because of the daily increase in number of susceptible root tips available for new infections. The same situation exists with *Heterodera schachtii*, as reported by Müller and Molz (28) and other European investigators of the trap crop for that nematode.

This experiment demonstrates a very heavy reduction in nematode population from a single planting of a trap crop. It likewise demon-

TABLE 2.—*Results of use of root galls of different ages as inoculum on root knot in subsequent plants. (Roots examined in every case after 30 days)*

Plants	Source of inoculum (See Table 1)	Age of oldest galls in inoculum	No. of galls developed on test plant
1	Lot 1 a, b, c	3	0
2	Lot 2 a, b, c	6	24
3	Lot 3 a, b, c	9	9
4	Lot 4 a, b, c	12	7
5	Lot 5 a, b, c	15	17
6	Lot 6 a, b, c	18	18
7	Lot 7 a, b, c	21	27
8	Lot 8 a, b, c	24	32
9	Lot 9 a	27	1,177
10	Lot 10 a	32	1,453

strates, however, that several trap-crop plantings are necessary in order to entirely eradicate nematodes from the soil. Even with the confined roots in the gallon cans used in this experiment not all the nematodes in 3 plantings and possibly not even in four were removed. The negative readings in most cases on the 4th planting would seem to indicate complete eradication. This conclusion is not completely justified, however, since in some cases negative readings in the third planting were followed by positive readings in the fourth. (Table 1).

Preliminary Tests of the Cowpea (*Vigna Sinensis*) as a Trap Crop in Soils of Known Initial Nematode Population

Experiments 2 and 3. In the previous experiment the initial nematode population of the soil was unknown. It was therefore possible to determine only the relative quantities of nematodes removed from the soil in each of the several plantings of trap crops. No means were provided for determining the absolute numbers of nematodes removed by each plant or the relationship between total numbers of nematodes removed and the original population.

Other experiments were conducted in which efforts were made to determine these relationships. In 2 such experiments, in which cowpeas were grown in gallon cans of soil and root-observation boxes (7), with known initial magnitudes of infestation, poor plant growth occurred, probably because steam-sterilized soil was used in order to eliminate chance nematode contaminations. The roots did not penetrate the soils so well as did similar plantings in the field. Consequently results were irregular and unsatisfactory, and this paper is not encumbered with a detailed account of the tests. Certain consistent conclusions are of interest, and serve to support similar conclusions from a final comprehensive test. They are incorporated in the conclusions therefrom.

Known Nematode Populations in 5-Gallon Containers of Soil Freed from Chance Contamination by Thorough Air Drying

Experiment 4. The final experiment of this series, installed primarily in connection with the studies on indicator crops reported elsewhere (12) and described fully therein, is included here only in so far as its results are pertinent to the trap-crop problem. Two series of 5-gallon (20-liter) jars consisting of 20 jars each were filled with soils previously freed from living Heterodera by thorough air drying (15) and inoculated with nematodes, 1 series with 50 larvae to each jar, and the other with 500. Cowpeas were planted and, on sprouting, were thinned to 1 plant per jar. Growth of the plants was good. The readings, on the entire root systems carefully removed after 30 days' growth, in numbers of galls and actual nematode content as determined by the clearing method (16), are summarized in table 3. This experiment was highly satisfactory in that it determined, with sufficient replications and low probable errors, the proportion of a known original population caught by a single planting of a trap crop under good growing conditions in a large volume of soil (approaching field conditions in range of root growth).

TABLE 3.—*The gall count and nematode catch in a single trap-crop planting in 5-gallon containers with known initial population*

Lot	Initial population	Nematode catch (Average of 20 plants)		Percentage catch
		Gall count	Nematode count	
A	50	10.55 ± .40	11.10 ± .41	22.2
B	500	131.8 ± 4.73	150.35 ± 7.63	30.07

DISCUSSION AND CONCLUSIONS

In the first experiment the heavy root systems of transplanted tomatoes were responsible for the better than 98 per cent catch of the total effective nematode population in the first of a series of 4 successive plantings. In 2 subsequent experiments cowpeas were seeded in containers of steam-sterilized soil inoculated with known numbers of nematodes, and obviously good conditions for plant growth were sacrificed to certainty of control of the population. Root growth was poor and clearly unable to reach the nematodes, and the results obtained are discarded. In a final test this difficulty was overcome by the use of sun-dried rather than steam-sterilized soil, which permitted of equally accurate nematode population control and at the same time good plant growth. In this case catches of 22 to 30 per cent of the original population were obtained in the original planting. The actual proportion of the "effective" population caught, however, may be

considered to be much higher than that indicated by these figures. This conclusion is based upon the results obtained from all the tests in which subsequent plantings were made (Experiments 1, 2, and 3) in which invariably all the later plantings have, together, caught much fewer than the original planting. The remaining nematodes, not accounted for in the root examinations of trap crops, may be considered to be in 1 or more of the following categories:

1. Those that have not found roots and that may do so later if a more extensive root system of a susceptible crop becomes available.
2. Those that have found roots but have failed to become established.
3. Those that have perished in the soil because of initial inherent weakness or some long-continued unfavorable condition of the environment (15, 17).

The tests indicate that class 1 accounts for only a small proportion, and it may be concluded that many larvae perish without producing infection.

The following conclusions are drawn from the trap-crop experiments: (1) A very high proportion, up to 98 per cent, of the total "effective" nematode population of soils in closed containers was caught by a first planting of a vigorous trap crop with a heavy root system, penetrating well through the soil. (2) Only from 22 to 30 per cent of total original populations were so caught in the first plantings, but the uncaught remaining populations were mostly "ineffective" in producing infection in subsequent plantings. (From tests 2 and 3 in part). (3) A considerably higher proportion of a higher population was effective in gaining entrance into root systems, than of a lower initial population, indicating some joint activity of larvae in penetrating roots (see 16, p. 328). This conclusion was consistent in tests 2 and 3 as well as in test 4. (4) In tests in which the larvae used for inoculation were placed in definite regions to one side of the container (test 3) only the roots in that immediate vicinity became infected, indicating a minimum migration of the larvae through the soil.

SUMMARY

The trap-crop (Fangpflanzen) method of reducing populations of plant-parasitizing nematodes, which during the past 50 years has received a great deal of attention in Europe in connection with the sugar-beet nematode, *Heterodera schachtii,* has occasionally been suggested for the control of *H. marioni,* but never has been adequately tested. Preliminary trap-crop tests in containers of limited capacity, in Hawaii, have shown that nematode populations can be very greatly reduced by one or more trap-crop plantings. Well-rooted tomato plants set in gallon cans of heavily infested soil and removed before the development of a new generation of nematodes, withdrew from the soil over 98 per cent of its effective nematode

population in a single planting. The roots removed from the infested soil and used to inoculate new cans of sterilized soil transmitted very low numbers of infective larvae up to the 27th day (under the conditions of the experiment), at which time heavy transmission took place due to the abundance of eggs at this stage of development. The limitation of root systems in this test to gallon cans undoubtedly made for a more complete catch than would occur in open fields. In another test cowpeas were planted as trap crops in 5-gallon containers of originally nematode-free (air-dried soils) inoculated with known numbers of nematodes. Twenty-two per cent were recovered with a single planting in the case of the low population inoculation (50 larvae), and 30 per cent in the high-inoculation series (500 larvae). In other similar tests, subsequent plantings of trap crops recovered only low additional proportions, showing that most of the uncaught larvae after first plantings were ineffective in producing infection. In the most intensive test, however, the third successive planting of a trap crop did not entirely eradicate the nematodes, and the fourth did not do so with absolute certainty, though remaining infestation was decidedly low.

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A STUDY OF THE ROOT-KNOT-NEMATODE TRAP CROP UNDER FIELD SOIL CONDITIONS[1]

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(Accepted for publication August 26, 1933)

INTRODUCTION

A companion paper from this Station[2] gives the results of greenhouse experiments with tomatoes and cowpeas as trap crops in small containers of soil. A review of the literature on the use of a trap crop for nematode control is given in that paper. A very high percentage reduction in nematode populations was obtained under the conditions of one experiment in a single planting of the trap crop. This was, of course, removed from the soil before the development of a new generation of nematodes. In 3 subsequent plantings the final ones still showed in some cases the presence of infestation.

In an effort to determine if, under conditions more nearly approximating those of the field, the trap-crop method might have a practical application, a further series of experiments was started at the Wahiawa field headquarters of this Station in the spring of 1930. Observations from a field rotation-crop experiment had shown, following 2 plantings of a trap crop, a very great reduction in nematode population of the soil as demonstrated by a later planting of an indicator crop. This reduction was clearly sufficient to be of significance in connection with the growing of a short-season annual crop. Its value for a perennial crop such as pineapple, was questionable, however, in view of later observations on the experiment referred to, which is discussed elsewhere in this paper. The great reduction mentioned from the 2 plantings, however, was encouraging as to the value of further intensive studies on the trap-crop proposition.

METHOD

A main desideratum of these new experiments was to approach field conditions as closely as possible in so far as type and depth of soil were concerned and yet to have the factors of uncertainty as to spread from one location to another completely eliminated.

This was accomplished by means of "isolated plots" like those used by

[1] Published with the approval of the Director as Technical Paper No. 61 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Godfrey, G. H., and Helene M. Hoshino. The trap crop as a means of reducing root-knot-nematode infestation. Phytopath. **24**: 635–647. 1934.

Byars[3] in Florida in his work with poisons. These consisted of 4 x 8 ft. galvanized iron bottomless boxes with sides 3 ft. deep inserted into the soil to a depth of 2 ft. The boxes were arranged end to end in rows, a single partition serving to separate adjacent boxes. They were riveted together at the corners, which were well painted with white lead to eliminate possible migration of nematodes from one box to another. The 2-ft partition below ground is practical insurance that no movement of nematodes from one box to another takes place in that region. The 1-ft. elevation of the sides above ground eliminates danger of surface spread by splashing, etc. The top soil was 10 inches deep. The final level of soil on the inside was approximately 2 inches on the average above that on the outside.

The area in which these boxes were placed had stood for years in elephant grass, *Pennisetum purpureus* Schumacher, a plant that is apparently immune from root knot. A test by means of indicator crops showed the initial nematode populations to be uniformly low. This presence of nematodes was probably due to the few susceptible weeds that grew in the field along with the grass.

The first step in the plan of procedure was to inoculate all plots for the experiments uniformly and heavily with the root-knot nematode. This was accomplished by inserting a uniform application of heavily infested cowpea roots. This being shown by test to be short of the heavy degree of initial infestation desired, additional inoculum was applied to each plot in the form of about 2 bushels of heavily infested soil from pineapple fields and the whole spaded under.

The main objective in the experiments to be conducted in these plots was, of course, to reduce the nematode populations of the plots as quickly as possible to zero, and at the same time to learn the utmost of what actually happens in the roots of susceptible plants when the latter are killed at different stages of nematode development. The proper time for removal of the plants was, naturally, an important consideration. The influence of season on length of life history of the nematode was recognized but at the time the experiments were installed the minimum length of life cycle of the nematode was not known.

Experiment 1: For this experiment it was decided to treat different plots as follows:

A Plots In duplicate. Susceptible crop (cowpea, *Vigna sinensis* L.) planted six rows in the plot, rows 6 inches apart, plants 4 to 6 inches apart in the row. At end of "x" days plants destroyed by turning under into the soil. In doing this a few individual roots were marked by means of strings and labels for later ob-

[3] Byars, L. P. Experiments on the control of the root-knot nematode, *Heterodera radicicola* (Greef) Mueller. Phytopath. 9: 93–103. 1919.

servation on the development of nematodes in decaying roots in the soil.

B Plots In duplicate. At the end of "x" days the plants were removed from the soil by so pulling as to get the major portion of the root systems. In this way it was expected that the comparison between A and B methods of plant destruction could be obtained. A few of the B roots were then thoroughly washed and placed in moist chambers for intensive study of further development of the contained nematodes.

C Plots In duplicate. Plants removed at end of "x + 1" days. Procedure otherwise same as with A.

D Plots Growing plants were left in the ground continuously. They were turned under at maturity and new plantings made; thus a continuous high nematode population could normally be expected.

E Plots Kept continuously fallow until eradication appeared to be complete in A, B, and C, when a crop was planted for purposes of comparison.

The first planting was made on April 10, 1930, and left, according to plan, for 23 days. It soon became evident that this period was too long, since laboratory examination of roots removed showed the development of egg masses. Such roots turned back into the soil would naturally constitute reinfestation. The succeeding few plantings were devoted to an effort to determine the maximum length of time plants could be left in the soil without bringing about an actual increase in the nematode population. The results of these efforts are shown in very compact form in the first part of table 1. The last column but one of this table shows the results in presence or absence of eggs from the laboratory examination of roots removed according to plan under the outline for A and B. It is obvious from the table that up to the end of the 6th planting, which was made on September 4, 1930, some reinfestation of the soil was taking place from roots that were returned to the soil.

Beginning on October 7, the plantings were continued, this time with assurance that plants would be removed in time to prevent such reinfestation. Special plantings of the same indicator plant in infested soil, made on the same dates as the trap-crop plantings, removed daily for examination near the approximate date, governed time of removal of the latter. An increase in length of cycle can be considered as evidence that, on the average, the soil was cooler than when the cycle was shorter. In other words, seasonal change in length of life cycle is taken into consideration. Observations were recorded only occasionally after this date, as shown by the continuation of table 1. On January 12, 1931, only 6 per cent of the plants

TABLE 1.—*A record of observations on trap-crop experiment No. 1*

Cycle	Date planted	Plots	Plants growing (days)	Infection			Development of eggs on roots after incubation period in	
				No. plants	Inf.	Eggs	Laboratory moist chambers	Indicator plants from the plots
	1930				*Per cent*			
1	4–10	A	23		100	−	++	
		B	23		100	−	++	
2	5–22	A	21		?	+++	++	+
		B	21		?	+++	++	
3	6–20	A, B,	19			+	+	+
		C	20			+	+	+++
4	7–16	A, B,	17				+	
		C	18				+	
5	8–11	A & B	15		+		−	+++
		C	16		+			+++
6	9–4	A & B	15					+
		C	16					+
7	10–7	A & B	20		+			
		C	21		+			
8	11–5	A & B	21		+			
		C	22					
9	12–9	A & B	21		+			
		C	22		+			
	1931							
10	1–12	A	28	235	6			
		B	28	236	2			
		C	29					
		D	29		100			+++
		E	60					+
	Continued through 12 more cycles, in length as follows: 11th, 24 days; 12th, 21; 13th, 18; 14th, 14; 15th, 13; 16th, 15; 17th, 16; 18th, 15; 19th, 18; 20th, 21; 21st, 28; 22nd, 21. Observations as follows:							
13	4–28	A	18	140	0			
		B	18	203	0			
		C	19	244	0			
14	5–29	A	14	182	14.3			
		B	14	183	13.7			
		C	15	202	8.4			
18	9–8	A	15	138	2.2			
		B	15	165	1.8			
		C	15	212	6.6			
	1932							
22	1–11	A	21	831	0			
		B	21	1618	0			
		C	21	1512	0			
		E	21	113	0			
23	2–18	A	60	92	1 plant	+		
		B	60	90	0			
		C	60	93	0			

removed from A plots showed infection and only 2 per cent of those from B. On this same date plantings were made in plots D and E. D plots showed very heavy infection, as might have been expected. E plots showed

only the slightest trace and that only after 60 days growth of the indicator plants. On April 28, 1931, in the 13th planting from the start of the experiment, but only the 5th after reinfestation ceased, the readings on all plants were zero. However, in readings subsequent to that, infection of plants began to appear again. It was not until the 22nd planting from the start, or the 16th after reinfestation ceased, that the readings on all plants were zero again. This zero reading was on January 11, 1932, approximately 14 months after reinfestation of the plots theoretically ceased.

In the entire tabulation the absence of a record means simply that no observations were recorded. Negative readings are not implied. After the second reading from the start of the experiment, infection was invariably very light, in most cases only 1 or 2 galls on each plant that was recorded as positive.

As a final check on this experiment another replanting was made on February 18, 1932, this to be left for 60 days. On April 18, 1932, these plants were removed for observation. Only a single plant in the entire lot, this in plot A, showed a single gall with one female and an egg mass. Thus, after $15\frac{1}{2}$ months of trap cropping with 16 successive plantings, at least one of the plots still retained infestation.

Experiment 2: An entirely different series of trap-crop plantings was made in another set of isolated plots starting on June 3, 1931. All plots were inoculated at the outset, by the addition of heavily infested pineapple soil. The soil was likewise enriched with decomposed pineapple waste to render conditions favorable for good plant growth. There were 3 treatments similar to those of Experiment 1, all in duplicate.

A Plots Plants turned under at end of "x" days.
B Plots Plants removed with major part of root system at "x" days.
C Plots Plants turned under at "x+1" days.

The length of the cycles were determined in part by previous experience on the basis of influence of season on length of life cycle, and, in part, by stage of development of galls on roots, as determined by observations on occasional plants.

The lengths of successive cycles, in days, were as follows: 1st, 14; 2nd, 18; 3rd, 14; 4th, 14; 5th, 15; 6th, 20; 7th, 21; 8th, 21; 9th, 23. With C plots the cycles were, of course, a day longer. It will be noted that here, too, the low-temperature cycles were of greater length in order to trap as many nematodes as possible before the first ones to infect the roots reached maturity. Notes on extent of root-knot development were taken on only a few of the successive trap-crop plantings, so observations are not tabulated. There were 9 successive plantings of trap crops. The first showed definite and relatively uniform but not extremely heavy infection. Subsequent observations showed only a trace; toward the end of the series only

occasional plants showed any infection, at all. The only observations recorded during the course of the series were as follows: 8th cycle, 22 days, ending January 4, 1932, C plots showed one plant only infected; 9th cycle, 23 days ending February 10, 1932, negative throughout.

Following this regular cycle, the entire series was replanted and left for 60 days. This gave time for deeper penetration of root systems to depths in the soil not reached by the shorter cycle plantings and likewise time for reinfestation from the progeny from any chance primary infection that may have occurred. The readings on this again showed a trace of infection in 4 of the 6 plots in the test. Two of these were from B plots and 2 from C plots, the A plots being zero throughout. Thus 9 successive plantings made through 8½ months did not completely eradicate the nematodes from the soil in most cases.

MISCELLANEOUS TRAP-CROP RESULTS FROM OTHER EXPERIMENTS

The Contribution of Trap Crops to Nematode Control in a Field Culture Experiment. During the period 1927 to 1930 a 4-acre pineapple field at the Wahiawa Field Station that was heavily infested with root-knot nematodes in the 1927 plant crop, was made available for a series of different intercycle culture operations with the objective in view of testing them both for effects on the nematode population of the soil and on pineapple yield in the planting to follow. The area was divided into 8 equal plots each measuring 75 by 300 feet, to receive 4 duplicated sets of treatments, A to D. Without explaining the experiment as a whole in detail, a trap-crop phase of one portion of it is here described because of its bearing on the subject-matter of this paper.

Two plots, C and D, both duplicated, had virtually the same set of treatments including, in order, a winter of a cover crop, a summer of clean fallow, another winter of cover crop and summer fallow, then nematode-resistant winter-cover crop again, and finally spring plowing and continuous clean fallow in preparation for fall planting (1930). The one outstanding difference between the 2 treatments was, for C plots, 2 plantings, in February and March, 1929, of English peas as a trap crop, one closely following the other. Both plantings were watched closely, with frequent examinations of roots, and were plowed under before time for the propagation of a new generation of nematodes. In February, 1930, the winter cover crop of pigeon peas, *Cajanus cajan* [L.] Millsp., growing in both C and D plots, was noticed to have harbored a heavy growth of weeds throughout the winter. The weeds in D plots known to be nematode-susceptible (principally *Emilia sonchifolia* L.) were abundantly, uniformly, and almost 100 per cent infected with nematodes. In C plots the same weeds were only scatteringly infected (about 10 per cent). Some

propagation of nematodes undoubtedly occurred in both sets of plots, since the weeds were growing a sufficiently long time to bring about such increase. Approximately the same order of difference in infestation persisted, however, throughout the balance of the preparation-for-planting period. An indicator planting of English peas in April showed a uniform, heavy infection for D plots. Another planting made at the same time in tubs containing composite soil samples from the different plots showed definite infection for D plots and no infection for 1 C plot with a bare trace for the other. The field was planted in November, 1930, to pineapples, according to regular plantation practice. Soil samples collected from the different plots the latter part of the same month and planted to cowpeas as indicators showed definite infestation present in both C and D plots, but distinctly greater in the latter. Final nematode observations were made on English peas planted in large numbers at regular intervals beside the pineapple rows in January and February, 1931, and removed for root observations about a month after planting. A summary of the results of this reading is given in table 2. A significantly lower degree of soil infestation is still manifest in the C plots 2 years after the trap crops were used.

TABLE 2.—*Summary of indicator crop readings of C plots (with trap crop) and D plots (without)*

Plot	Number of plants		Plants infected
	Infected	Not infected	
			per cent
C–1	175	3166	5.2
D–1	1358	1704	44.3
C–2	394	3288	10.7
D–2	1719	1888	47.6

Consideration of all the results in nematode readings on this experiment would appear to justify the conclusion that the trap-crop plantings definitely contributed to nematode reduction. Since there was nematode survival of a relatively low order throughout, however, it is not likely that the difference brought about by the trap crop is sufficient to make itself felt significantly in the pineapple planting. Unfortunately, insufficient experiments have been conducted with single-season crops to form an adequate basis for judgment as to the value of the extent of reduction obtained when such crops come into consideration. We have only the general information from the indicator-crop studies previously referred to (Ref. 12, other paper), that a high percentage of indicator plants showing infection means

a relatively high degree of soil infestation, and one that will make itself manifest on the growth of a susceptible crop planted immediately; and on the other hand, that a low percentage of such plants showing infection means relatively low infestation of the soil and in all probability one that will permit such a crop to grow through a season without serious injury. Further experiments are needed on the common susceptible annual crops, both to determine the amount of injury obtained from known initial infestations and to determine the practicability of the use of the trap crop to reduce infestation to noninjurious proportions.

Spraying with a Weed Killer to Destroy Trap Crops. In July, 1930, 5 tubs of soil heavily infested with nematodes were planted to cowpeas. Seventeen days after planting, the plants in 3 of the tubs were sprayed with sodium arsenite, containing 5 pounds arsenic to 100 gallons. The spray was completely effective in killing the aerial portions of the plants. A week later the nonsprayed plants showed continued development of the nematodes within the roots, and egg masses with eggs were evident. In the sprayed plants such continuation of development did not occur. Nematodes could be found within the roots in the early pyriform stage, without sign of egg masses. In another week roots were well decayed. The spraying had effectively killed the plants and had definitely resulted in the destruction of the nematodes that were caught in the roots and prevented the propagation of a new generation. Repetitions of the experiment produced the same result. Observational sprayings on weeds and indicator plants under field conditions likewise brought about efficient killing of the plants.

These indications of the practicability of poison sprays in connection with the use of trap crops are in line with the contributions of Müller and Molz in Europe, referred to in the other paper (Ref. 28). The work with poison sprays for killing trap crops, planned for large-scale field application, was not continued owing to the press of other activities. A study of the extensive literature on weed killing indicates, however, that a number of different sprays would be entirely practicable for the purpose in mind, should trap crops be used for nematode reduction on any large scale.

An Attempt to Reduce Nematode Population in the Pineapple Row by Trap-crop Plantings. In 1927 and 1928 susceptible soybeans and cowpeas were planted close beside the rows of extremely heavily nematode-infected young pineapple plants with a view to determining if repeated planting and removal of such trap plants would reduce the extent of pineapple infestation. A first planting of soybeans, made in late August, was extremely heavily infected, and each plant definitely removed from the soil thousands of nematodes. Thereafter throughout the winter and until June of the following year repeat plantings were made along the same rows, and removed always before the maturation of the nematodes. Each planting was

compared in degree of infection with a first planting in a near-by row. Until the sixth planting no marked differences were evident, practically all plants alike showing medium or heavier infection. From that time on until the 11th and final planting of the test the repeat plantings showed some reduction in degree of infection as compared with the first plantings; but, even in the last planting, infection was definitely present in most of the plants. Table 3 shows the course of the nematode readings obtained throughout the test, in terms of index figures representing degrees of infection, from 0 to 5. Since relative infections only are relevant, the exact number of galls for each index figure is not necessary, though roughly, the number 5 may be considered to represent about 1,000 galls per plant and each digit below that about 200 less.

TABLE 3.—*A record of degree of nematode infection on a series of trap-crop plantings beside the pineapple row. (Scale 0 to 5)*

Plantings	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th
"Repeat" plantings	5	3	4	3–5	4	2–3	1	.....	2	2	0–4
"First" plantings for comparison.....		5	4	5	5	2–3	1–5	.....	4	4	0–5

Pineapple plants removed at random from the rows receiving the influence of the 11 trap-crop plantings showed no visible reduction in nematode infection as compared with near-by rows, and no effect whatever on plant growth was evident. It would appear that the soil around the pineapple plants had already, at the start of the test, reached the "saturated" condition in so far as nematodes were concerned, and that only a surplus of nematodes that might otherwise largely have perished were caught in the trap plants from the limited range of soil reached by their roots. The nematodes in the pineapple root systems were constantly propagating new generations and thereby maintaining a supply sufficient to infect the new roots as fast as they entered the ground. The trap crop in this case was apparently of no value to the pineapple plants.

DISCUSSION

The detailed observations on the stage of development of nematodes at the time of removal or destruction of trap plants in the first experiment herein reported make it evident that the presence or absence of the initiation of an egg mass is not a safe criterion on which to base judgment as to the chances for further propagation of nematodes if the roots are left in the soil, even when they are detached from the plants. In the course of development of the female there would appear to be a critical point in the

early pyriform stage before which it probably will die if the plant is killed and after which it may eventually attain maturity and deposit at least a few eggs. Miss Tyler, in her pure culture studies (Previous paper, reference 34, p. 378) has shown that the nutritional requirements of the nematode may be met without a growing top to the plant at any stage of nematode development. She has carried the nematode through its complete life cycle, from infection of the root to maturity and egg development, in small detached roots. The decay of the roots, then, before eggs are produced, must be considered as the ultimate factor that prevents continued development and the propagation of a new generation. From this necessary point of view, conditions favoring the rapid decay of the roots of the trap plants are an important adjunct to the successful use of the trap-crop method. This is a soil-management problem, and involves the assurance of the presence of sufficient soil bacteria of the right sort, and of nitrogen and phosphorus, and moisture and temperature conditions favorable for microbiological activity.

The conclusion is obvious from this series of tests that the trap crop may be of value only in quickly reducing heavy soil infestation to a relatively low point. It is decidedly not indicated for nematode eradication. Under field conditions the roots do not ramify through the deeper layers of soil, where the organisms may be expected to survive longest, sufficiently well during the limited period that is allowable, to catch them all in the 2 or 3 plantings that would be practicable. Older plants, 60 days or more in the ground, will reach more of them, but this period of growth is so long that the earlier infections are almost certain to produce a new generation of larvae and thus defeat the purpose of the trap crop. While the use of trap crops has been unpromising as an *eradication* measure, it has definitely brought about high percentage *reduction* of nematode population in 1 or 2 plantings only. This feature of the results of these trap-crop studies, if judiciously applied, may prove to be of practical value *in connection with* other means of nematode control.

SUMMARY

Further studies with trap crops for the reduction of soil population of the root-knot nematode, *Heterodera marioni* (Cornu), were conducted under conditions approaching those of the field. The plots used for the experiments were of normal field depth and were separated from one another, to avoid cross contamination, by means of galvanized iron sheets 2 feet in the ground and 1 foot aboveground. In 2 tests, cowpeas (*Vigna sinensis* L.) and English peas, *Lathyrus sativus* L., both highly susceptible to root knot, were planted as trap crops in a succession of several plantings. In the trapping tests proper they were removed from the ground or

turned completely under, always before the development of a new generation of nematodes. Thus, every planting destroyed some of the original nematode population. In neither of the tests was a final zero-point infestation reached in all plots, even after, in the one case, 16 successive plantings during nearly as many months, and in the other case 9 successive plantings. A bare trace of survival occurred in both cases. Nematodes in the deeper layers of soil were not reached by the roots during the short period of growth (14 to 24 days, depending upon the temperature) necessary to insure that no reproduction would take place. No better results in final eradication were obtained by this method than were obtained by plots maintained under completely fallow conditions during the same period. For complete eradication, therefore, the trap crop is not to be considered. Two plantings, however, reduced infestation by a high percentage (upwards of 90 per cent), so that, judging by general knowledge of the growth of plants in nematode-infested soils, a susceptible annual would survive the season without serious injury.

A test in a heavily infested young pineapple field gave substantial proof that a long succession of plantings of trap crops beside the crop row was not capable of reducing the nematode population sufficiently to become obvious in the pineapple plants. Other tests indicated the practicability of the use of poison sprays for the killing of trap crops at the right stage, under field conditions.

IS PSOROSIS OF CITRUS A VIRUS DISEASE?[1]

H. S. FAWCETT[2]

(Accepted for publication August 21, 1933)

A symptom of psorosis expressing itself as a mosaic-like effect on young leaves has recently been discovered (6). This, taken in conjunction with information that has been accumulating for a number of years, suggests the probability that it is a virus disease. It is the purpose of this paper to present the results of experiments and observations that bear on this phase of the subject. More evidence is needed to prove beyond question the virus nature of the trouble.

The histology of tissue affected with psorosis, as compared to normal tissue, will form the subject of another publication by Irma E. Webber and the writer.

The characteristic effects of psorosis heretofore observed on the trunk and branches have been described in detail in previous publications (3, 4). In brief, one of the first evidences on the bark is either small scales of bark pushed up from the surface and attached by one edge, or small, pimple-like pustules or eruptions (Fig. 1). These usually start at some small localized region and enlarge slowly to form large areas. It is not uncommon for only one area to be seen on the trunk or large limb at first. Frequently, however, a number of small areas will become visible at the same time, and these by enlargement and merging form large areas encircling the trunk or a large limb in one season. Drops of gum are later exuded.

Another symptom (5) first makes its appearance as a drop or drops of exuded gum, then the bark begins to scale in much the same manner as the other, but usually with a more rapid spread and a more rapid continuous extension along branches to smaller and smaller twigs. Later, effects of both forms show continued scaling of outer bark and gum formation, not only in the bark elements, but also in the region of the wood. Frequently, the final effect after some years is a staining of the deeper layers of wood in the trunk and larger limbs and either a slow or rapid decline of the tree.

The hypothesis that the causal agent of psorosis is a microscopic organism, such as a fungus or bacterium, has directed investigation of this disease for many years. Such a theory of the disease had appeared logical because of the definite, localized scaly bark lesions. These start, in the beginning stages, in the outermost layers of bark and spread slowly in surrounding tissue, and only later affect the deeper tissues of the bark, cam-

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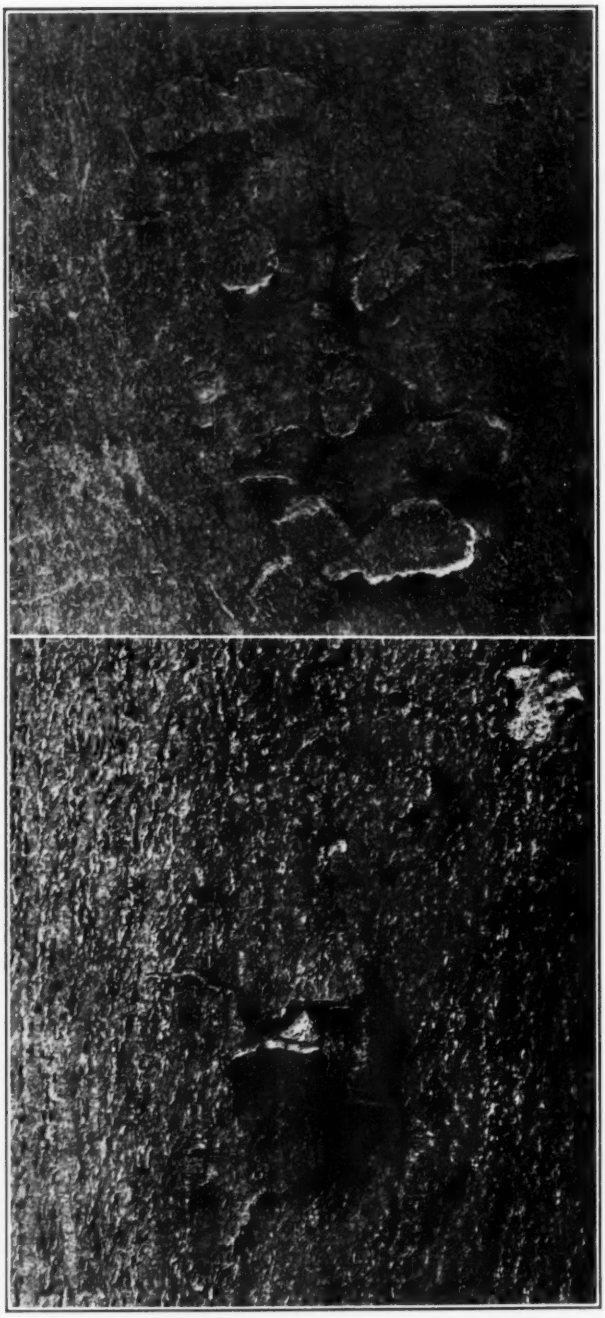

FIG. 1. Beginning stage of psorosis on the bark, showing formation of scales pushed up from the surface. About × 1.

bium, and wood. Moreover, the manner of development of these lesions is not unlike that of other bark diseases, the causal agent of which was known. In fact, shell bark of lemon (2), now known to be due to the fungus *Phomopsis citri,* is somewhat analogous to psorosis in the peeling off, in scales, of outer layers of bark, while the inner bark and cambium remain alive. In a few instances, 4 cases out of 100 or more trials, psorosis has been transmitted to healthy orange trees by placing under the bark pieces of tissue from diseased lesions. In these cases 2 to 8 years elapsed before the expression of the disease was noted on the bark of the trees inoculated. In an attempt to determine the causative agent, many species of organisms have been isolated in past years from the superficial tissues of psorosis bark lesions, but inoculations have all failed to reproduce the disease.

The symptoms associated with psorosis, which have not been previously described in detail, are mosaic-like effects on young leaves and clear, more or less circular spots on older mature leaves, spots on water sprouts, and rarely on fruit.

Mosaic-Like Effects on Young Leaves

This condition was noticed for the first time on May 15, 1933, at Riverside, California, on the same Valencia orange trees from which buds had been taken for propagation in 1922, as described later. The mosaic-like appearance is found to be produced by many small, elongated, light-colored areas usually in the region of the smallest veinlets of the leaf blade. The effect appears to be due not only to a clearing of the veinlets themselves, but to a clearing in tissue just adjacent to the veinlets, as well (Fig. 2). These small, cleared places usually are very numerous and either scattered over the entire blade or occupy only certain portions of it. When pronounced, the effect gives a stippled appearance to the small young leaf blades when held to the light. As the leaves become larger and approach maturity, the stippled effect is masked. The areas that produce the stippled or mosaic-like effect vary much in size. Spots are often about 1 to 3 mm. long and $\frac{1}{2}$ to 1 mm. broad. Sometimes they are about the same length and width, and, when only 1 mm. or smaller in diameter, give the appearance to the unaided eye of being more or less circular dots. Often the pattern is not distinct and the spots fade out gradually at the margins or run together. In very small young leaves an appearance of vein-clearing alone is sometimes seen. They appear to be more pronounced in young leaves on rapidly-growing shoots or water sprouts than on leaves of slower-growing shoots.[3]

[3] Since this article was submitted, attention has been called to a mosaic-like variegation of sour orange leaves in Sicily, described by L. Petri, who suggests that it is transmitted by an aphis *Toxoptera aurantii.* Judging from Petri's illustration and description, it is believed to be different from the mosaic-like symptom of psorosis in California

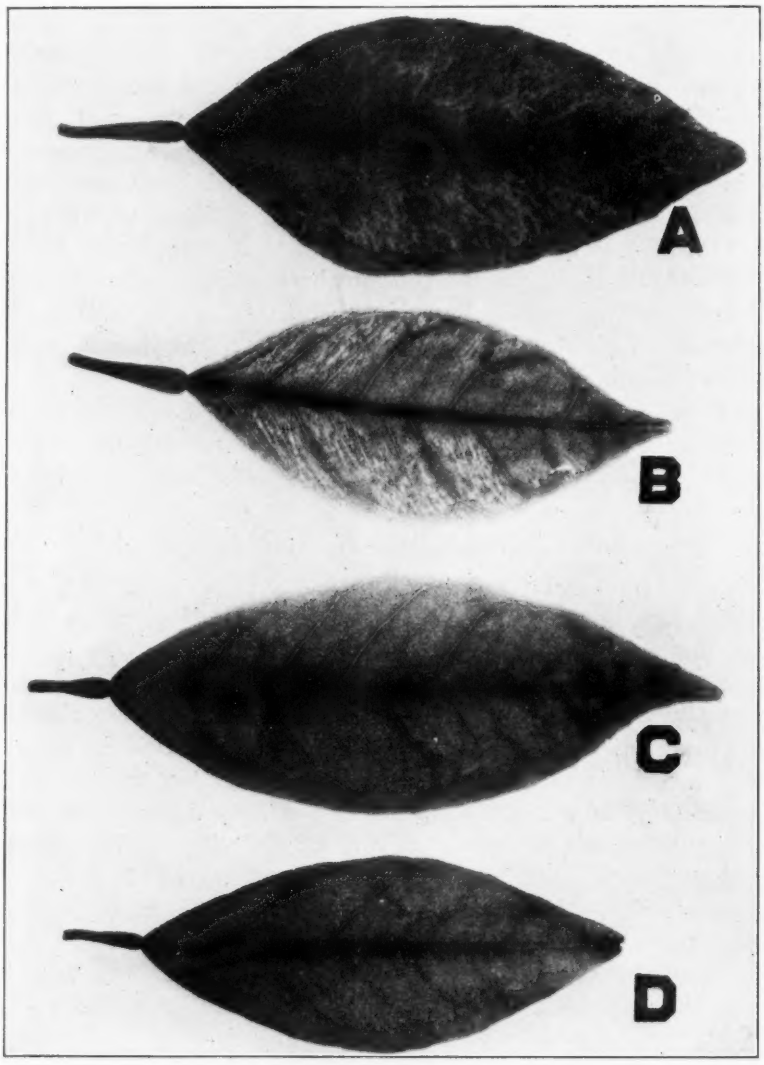

FIG. 2. Immature leaves of Valencia orange. A and B. Mosaic-like symptoms by transmitted light. C and D. Healthy leaves. About × 1½. Photographed by L. J. Klotz.

Spots on Older Leaves, Water Sprouts, and Rarely on Fruit

The spots on older leaves that had been noted occasionally for some years past vary in size and appearance from mere dots to larger, translu-

on sweet orange. Unlike the mosaic of psorosis, the one described by Petri is accompanied by definite warping and crinkling of the leaves and appears to depend on the previous presence of aphis on the leaves affected.

Petri, L. Variegatura infettiva delle foglie di "Citrus vulgaris" Risso. Boll. R. Staz. Patol. Vegetale **11**: 105–114. 1931.

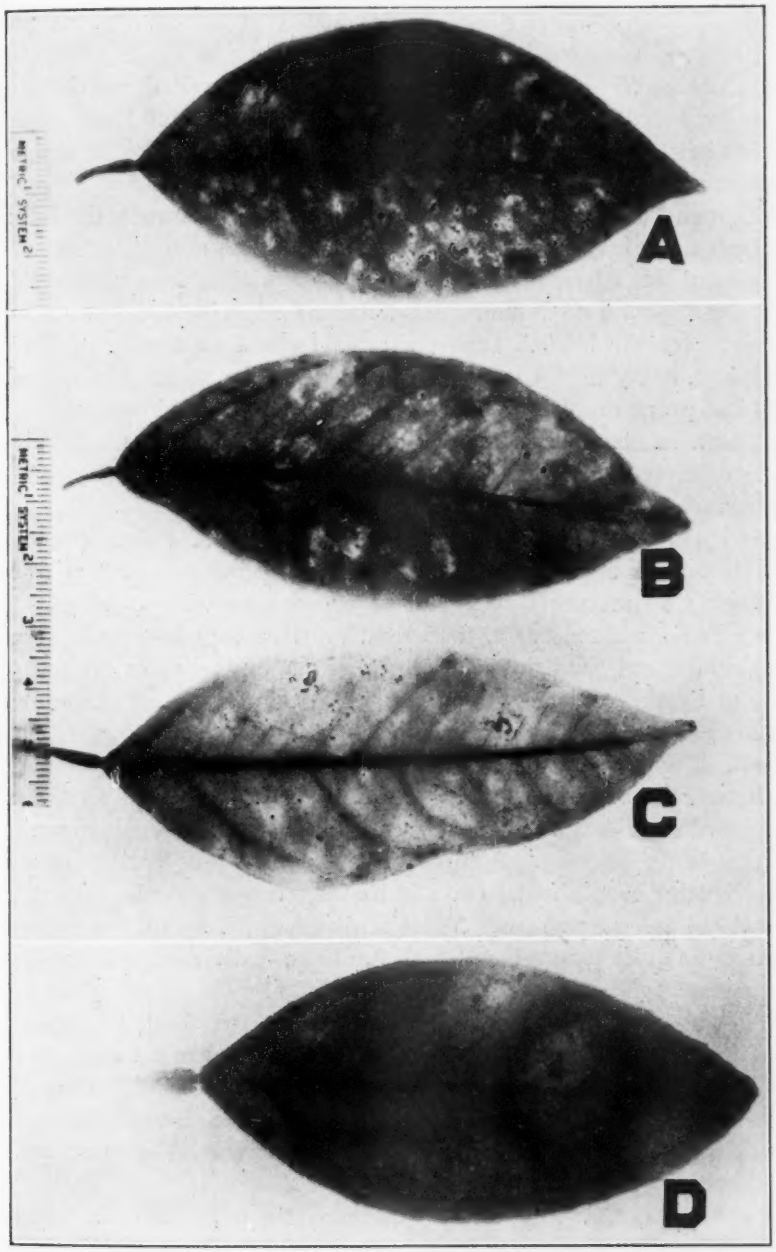

FIG. 3. Mature leaves of Valencia orange with light spots associated with psorosis. In A and C raised corky rings show in the light areas. About $\times \frac{3}{4}$. Photographed by L. J. Klotz.

cent areas more or less circular and frequently in the form of rings (Fig. 3). Corky, raised areas, forming either a pustule in the center, or rings or partial rings, are common in these spots. This raised, darker appearance is produced by greater cell division below and deposition of certain products in some of the layers of cells near the surface. Few or many such spots occur on a single leaf.

Although these spots vary greatly in size and appearance, the following actual examples may be mentioned. Some Valencia orange leaves on trees with psorosis bark lesions showed, in July, circular to irregular, yellowish, translucent spots ½ to 7 mm. across, not raised, with indefinite margins, scattered over the blade. Others showed spots 1 to 6 mm. in diameter, with raised, corky dots ½ mm., in the center on the upper surface. Other leaves had pronounced irregular ring spots 5 to 10 mm. in diameter. One area 8 mm. in diameter had 2 corky concentric rings, the smallest 1 to 2 mm. in diameter, and the largest 4 mm. with a yellow halo beyond. The rings are often not complete.

The spots that occur occasionally on the bark of water sprouts that originate from a badly diseased trunk or large limb resemble at first, the raised spots on mature leaves, and occur with or without rings. Later, as the water sprout matures, the raised portions may become more corky, glazed, and hard, and in some cases resemble the spots so commonly produced by the presence of leprosis (4). On only three different occasions has any effect on fruits been noted that might have a connection with psorosis. One of them was 2 or 3 grapefruits with depressed, circular furrows borne on a branch affected with psorosis. Another was a similar, less pronounced circular furrow seen recently on a single Valencia orange fruit on a tree badly diseased. A third was a Navel orange fruit with a dark-brown circular band about 10 mm. in diameter on a tree with an extremely bad and old case of psorosis. These fruit effects, if symptoms of psorosis, appear to be quite rare. Usually all fruits on badly diseased trees appear to be normal.

Following a careful study of these symptoms on both young and old leaves, it has been possible to find them either in a pronounced or inconspicuous form in association with a large majority of badly affected cases of psorosis on both Valencia and Navel orange trees. However, many trees with psorosis on the bark in a less severe form have been examined with no visible leaf symptoms.

It is believed that psorosis may occur on the bark either as a localized form, without being systemic until it has been on the tree for a number of years, or that it may be transmitted by budding in a systemic form and express itself on the bark after the tree is 6 or more years of age.

This hypothesis of systemic and localized forms is based not only on the

observations mentioned above but on the results of treatment of bark lesions in their beginning stages. Many trees appear to have been cured of psorosis by either scraping off the outer affected bark or by cutting away the bark over the diseased portion. On other trees the disease appears to have been eliminated by cutting off a certain branch that was first affected. At present, however, the data are not sufficient to establish with certainty this hypothesis.

Transmission by Buds

On April 28, 1922, bud sticks consisting of small twigs just back of fruit were taken from 5 Valencia orange trees on which there were bark lesions farther back on the larger limbs or on the trunk. The twigs used as a source for budding were not visibly affected with bark lesions. If leaf symptoms were present they were entirely overlooked.

A similar set of bud sticks was taken from five other trees that at the time showed no visible bark lesions of psorosis. Three of these 5 trees, however, afterwards developed bark lesions, leaving only 2 on May 15, 1933, that were free from psorosis. Buds from all of these were placed in sour orange nursery trees on May 3, 1922. The trees were set out in orchard form on May 5, 1924. They were examined from time to time and no differences were noted until the summer of 1931 when some trees began to show one or more small inconspicuous lesions on the bark of the trunk or older branches. This was 9 years from time of budding.

The mosaic-like symptoms had not been discovered at that time, so no data as to this effect are available. On May 15, 1933, the mosaic-like symptoms were discovered on the parent trees having psorosis. The next day a survey of the experimental plot gave the results shown in table 1.

It will be noted that all of the trees originated by buds from trees with visible psorosis in 1922 showed the mosaic-like symptoms in 1933 on young

TABLE 1.—*Experiments in transmission of psorosis of Citrus by budding*

Condition of parent trees	Number of trees propagated	Number of trees on which symptoms were apparent on May 16, 1933		
		On young, immature leaves	On mature leaves	On bark
Psorosis, 1922 Psorosis, 1932	23	23	20	14
No psorosis, 1922 Psorosis, 1932	17	9	6	4
No psorosis, 1922 No psorosis, 1932	11	0	0	1

leaves, and 14 out of 23 trees showed symptoms on the bark. It is believed that the parent trees that showed psorosis in 1922 had the systemic form and, therefore, transmitted the disease through the buds, and that all of the progeny will eventually show it on the bark. In most cases of psorosis in commercial orchards the bark lesions are not apparent until the trees have been set out for from 8 to 10 years, and usually even longer.

About one-half, or 9 out of 17, of the progeny from the parent trees that showed no psorosis on the bark in 1922, but had developed bark symptoms by 1932, show the mosaic-like leaf symptoms. Four out of these 9 show the bark symptoms. It is not known when this group of parent trees first showed bark symptoms, but the extent of the diseased areas in 1932 indicated that the symptoms had been present for a long time. If psorosis was carried by the buds, at least part of these buds may have been permeated in 1922 by the psorosis-producing principle, even though no bark symptoms were then evident on these parent trees.

None of the trees originating from the 2 parent trees free from visible psorosis in 1922 and still free in 1932 showed any mosaic-like leaf symptoms or mature leaf symptoms in May, 1933. One tree, however, in this group showed bark symptoms. It is believed that this tree may not have had the systemic form and may have become infected from the outside since it was budded. This would agree with the hypothesis that the disease may exist either as localized lesions and only later becoming systemic, or that it may be systemic from the first by being transmitted by budding from a parent tree that already had the systemic form.

Experiments with Rooted Cuttings

In December, 1926, F. F. Halma rooted some leafy shoots from a Valencia tree about 20 years old, showing a severe case of psorosis on the older bark. The bark lesions had been noted about 7 years before, when the tree was about 13 years old. The shoots themselves appeared to be healthy and nothing abnormal was noted at the time. Other shoots from healthy trees were rooted at the same time. These were set out as small trees in 1929. In June, 1933, the trees originating from a psorosis-infected tree showed the mosaic-like symptoms on young, immature leaves, and clear spots on older leaves, but no bark symptoms. Trees originating by rooted cuttings from a healthy tree in the same orchard were free from these effects. The same symptoms on leaves were found on young Navel trees propagated in March, 1928, by means of rooted leafy shoots taken from a tree badly affected with psorosis, while those similarly propagated by means of leafy cuttings from healthy trees showed no symptoms.

Since the discovery of the mosaic-like symptom, a series of experiments has been started on the basis of the virus hypothesis to determine more

definitely the mode of transmission and development of the disease. Inoculation experiments in collaboration with L. J. Klotz and experiments in budding, bark grafting, etc., have been initiated. Having learned to recognize the mosaic-like symptom, it has already been possible to recognize after only 5 weeks the symptom on the new shoots that have grown out from the diseased buds placed in healthy nursery stock.

The general resemblance of some of the symptoms of psorosis (especially the effects frequently found on mature leaves and water sprouts) to leprosis and ring-blotch lesions suggest the possibility that these may be in the same class of diseases. The nature of the cause of neither of the latter two diseases has so far been satisfactorily explained. E. M. Doidge (1) has previously suggested, among other hypotheses, the possibility of a virus for the ring blotch in South Africa.

SUMMARY

A symptom of psorosis appearing as a mosaic-like effect on young leaves was discovered in May, 1933. This is found most distinctly on young leaves of rapidly growing shoots. The mosaic-like effect is produced by small, light-colored areas in the region of the smallest veinlets. Symptoms on the mature leaves are light-colored, translucent spots often with raised, corky centers or with raised rings. Frequently, raised spots are found on the bark of water sprouts. Circular bands or furrows on fruit, probably associated with psorosis, have only rarely been found.

The previously well-known conspicuous symptom, which was evidenced by localized areas of scaly bark, had heretofore led to the hypothesis that the cause should be sought for in a microscopic organism.

The finding of these mosaic-like effects on leaves, together with the transmission of the disease by budding and by rooting leafy shoots, suggests a virus origin for the disease.

Experimental and observational evidence is given for the suggested hypothesis that the disease may occur either in a localized form restricted to a portion of the bark or in a systemic form spread throughout the tree to the buds, leaves, and other parts. It is suggested because of the general character of some of the lesions of psorosis on leaves and water sprouts to those of leprosis and ring blotch that these three diseases, though distinct, may belong to the same class of diseases.

CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

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THE ASSOCIATION OF CERCOSPORELLA HERPOTRICHOIDES WITH THE FESTUCA CONSOCIATION[1]

RODERICK SPRAGUE

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INTRODUCTION

Cercosporella herpotrichoides Fron is a soil-borne fungus, which causes a foot rot of winter wheat, *Triticum vulgare* Vill., and winter barley, *Hordeum vulgare* L., in certain prairie areas in Washington, Oregon, and Idaho (2, 3, 4, 5, 6). While there is small likelihood that the disease will become general throughout the wheat-growing sections of these States, the fungus causes severe losses in certain areas. The following data on its ecological associations are presented.

THE NATIVE VEGETATION OF CERCOSPORELLA-INFESTED AREAS

The vegetation of the prairie regions in the Columbia Basin largely belongs to what Weaver (7) termed the Agropyron-Festuca association. He divided this association into two consociations, the semi-arid bunch grass or Agropyron (dominant) consociation on the one hand and the more humid region sod-grass or Festuca (dominant) consociation on the other. Weaver's type area was Whitman County, Washington; the dry western portion belonging to the Agropyron consociation, and the somewhat moister eastern half to the heavier sod-grass or Festuca consociation.

Range stock and the plow have made heavy inroads on the native vegetation of the Cercosporella-infested localities, but there remain enough unmolested portions to determine the native flora with a fair degree of accuracy. Most of the Cercosporella foot rot occurs on Festuca sod-grass prairies (Festuca consociation) very similar to those studied by Weaver, except that most of the soils are somewhat lighter than those of the type area in the Palouse Region of Whitman and adjacent counties. A discussion of the native flora of the several Cercosporella-infested areas follows.

Columbia Gorge Area

Tygh Ridge, Wasco County, Oregon.—The Cercosporella-infested fields lie on a rolling, dissected plateau country, just east of the conifer-covered Cascade range, at an elevation of 2,700 feet, and within sight of the Columbia Gorge. Scattering trees and shrubs occur on the slopes below the wheat fields, but unmolested portions of the hill tops are covered with a

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations.

Festuca sod-grass consociation. In this consociation *Festuca idahoensis* Elmer (dominant),[2] *Poa sandbergii* Vasey, and *Agropyron* spp. are the more prominent grasses. In the drier locations clumps of *Agropyron spicatum* (Pursh) Scribn. and Smith are more prominent, and on rocky outcrops occur still more primitive, scattered grass associes. In sod-grass bordering foot-rot-infested wheat fields certain spring flowering plants occur in considerable numbers. Among these *Tellima tenella* (Nutt.) Walp., *Lomatium geyeri* (S. Wats.) Coulter and Rose, *Fritillaria pudica* (Pursh) Spreng., and *Brodiaea grandiflora* Smith are prominent during the spring months. In late spring and summer *Paeonia brownii* Dougl., *Balsamorhiza deltoidea* Nutt., *Astragalus* spp.,[3] *Lupinus leucophyllus* Dougl., *L. laxiflorus* Dougl., *Eriogonum compositum* Dougl., *E. sphaerocephalum* Dougl., *E. heracleoides* Nutt. and *Eriophyllum lanatum* (Pursh) Forbes, dominate the flora.[4]

Seven Mile Hill, Wasco County, Oregon.—This Cercosporella area is on a wind-swept prairie 1,500 feet above the bottom of the Columbia Gorge (fig. 1, A and B). The prairie was originally covered with an ill-defined sod-grass consociation in which the flora was similar to that on Tygh Ridge. Prominent flowering plants growing adjacent to foot-rot-infested fields include *Sisyrinchium douglasii* A. Dietr., *Fritillaria pudica, Delphinium menziesii* DC., *Balsamorhiza deltoidea,*[5] *Eriophyllum lanatum, Astragalus hoodianus, Lupinus caudatus,* Kellogg, *L. leucophyllus* and *L. laxiflorus.* There are thickets of Oregon oak, *Quercus garryana* Dougl., on thin soil portions of the prairie, which are bordered by the following shrubs: *Symphoricarpos racemosus* Michx., *Ceanothus integerrimus* Hook. and Arn., *Opulaster opulifolius* (L.) Kuntze and *Rosa* spp. There also are scattered wind-beaten red-fir trees, *Pseudotsuga mucronata* (Raf.) Sudw., encroaching from the near-by Cascade Range (figs. 1, A and B). Clements (1) illustrated a portion of this prairie as typical of the Agropyron-Festuca association.

[2] *Poa sandbergii* is now dominant in moderately grazed areas; *Festuca idahoensis* Elmer and to some extent *Agropyron* spp. disappear under grazing. In over-grazed areas, *Bromus tectorum* L. replaces most of the native grasses. This holds true for Cercosporella-infested areas.

[3] *Astragalus hoodianus* Howell is common on High Prairie, *A. conjunctus* S. Wats. on Tygh Ridge, and *A. reventus* A. Gray in the far eastern part of Oregon and Washington.

[4] Phenological observations show that the Cercosporella disease on Tygh Ridge becomes evident first as *Lomatium geyeri* comes into bloom; is well started when *Fritillaria pudica* blooms; and, under average seasonal conditions, is advancing most rapidly when the mass of spring flowering plants are making their greatest show.

[5] The *Balsamorhiza deltoidea* of the Columbia Gorge region differs considerably from the typical species west of the Cascades. It may be a transitional species between *B. deltoidea* and *B. sagittata* (Pursh) Nutt.

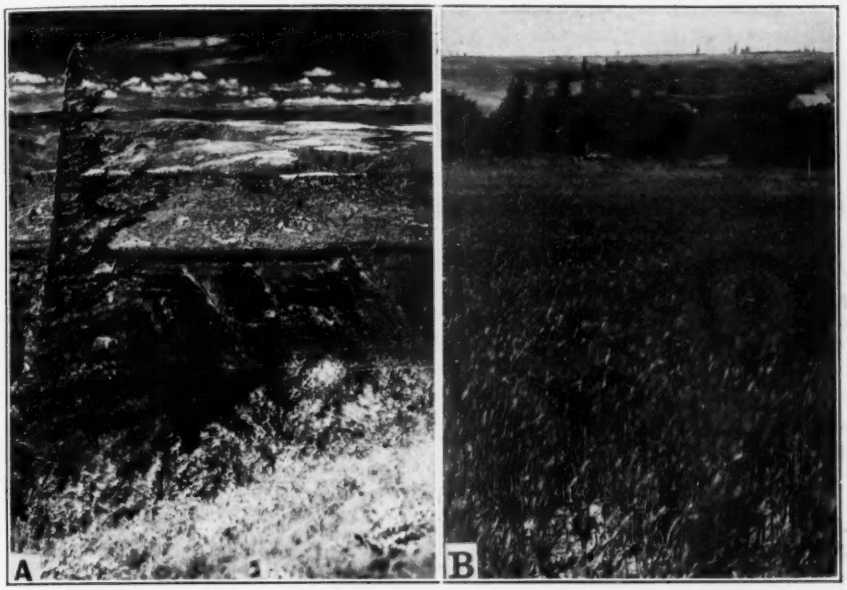

FIG. 1. A. View of Columbia Gorge with sod-grass prairie of Seven Mile Hill, Ore., in foreground and wheat fields on High Prairie, Wash., across the Columbia River in distance. Note directions of prevailing winds (up stream) as indicated by "wind timber" in foreground. Photograph taken with Eastman red-sensitive plate. B. General view of Seven Mile Hill, Ore. Most of the open prairie areas are now seeded to grain. The thickets of Oregon oak with scattered red firs are shown. The edge of the Columbia Gorge is outlined in the distance by "wind timber" red firs.

The Cercosporella foot rot sometimes occurs in mild form several miles westward from the type area in the transition territory between the grassland and fir-forest climaxes. This territory is characterized by a mixture of deciduous trees and second-growth red fir.

High Prairie, Klickitat County, Washington.—This Agropyron-Festuca prairie lies at an elevation of 1,600 feet and is a dozen miles, air line, across the Columbia Gorge from Seven Mile Hill (fig. 1, A). The prairie is nearly treeless, the surrounding slopes being covered with trees or bare, depending on exposure. A few miles east, in a treeless region near Goldendale, Washington, a few minor foot-rot infestations occur during average seasons in moist swales of isolated fields.

Most of the original vegetation on High Prairie was of the Festuca consociation (fig. 2, A) resembling that of Seven Mile Hill. Scattered spots of a soil phase whiter than the predominating type (possibly subsoil outcrops) and poor in organic matter, are nearly free from foot rot. This type of soil, which, on virgin lands, bears an Agropyron consociation, also, produces a poorer crop of grain than do the darker soils of the Festuca consociation.

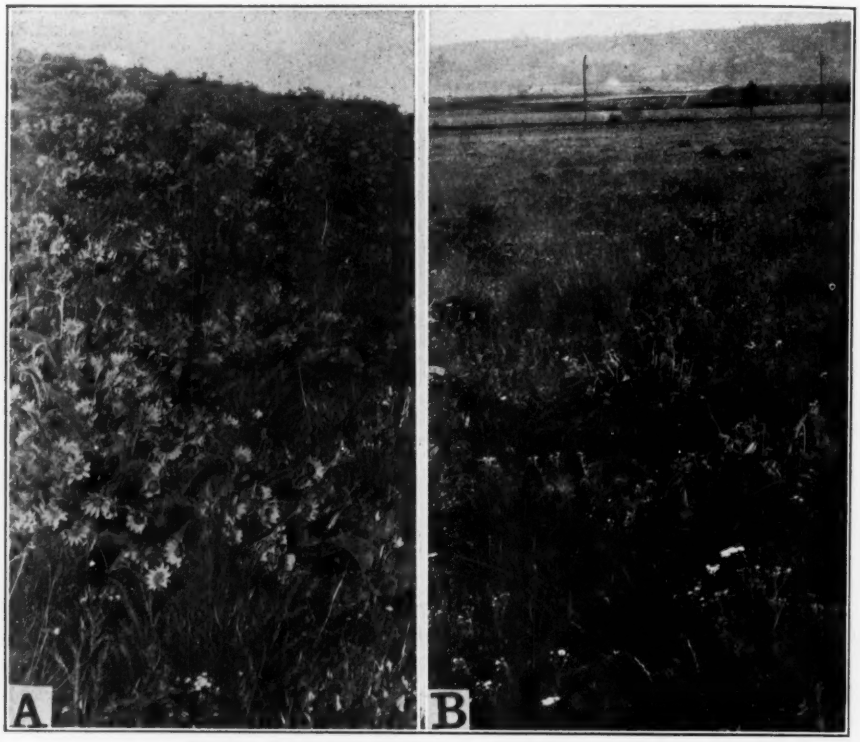

FIG. 2. A. *Balsamorhiza deltoidea* associe in full bloom on High Prairie, Wash. B. Prairie near Liberty Lake Junction, Wash., adjacent to Cercosporella foot-rot-infested field. Clumps of *B. sagittata* are prominent in the center foreground. Potentilla, Gaillardia, and Achillea also are prominent.

Grande Ronde Valley Area

This area lies in northeastern Oregon (Union County) about 200 miles east of the Columbia Gorge area. The comparatively level valley, surrounded by the conifer-blanketed Blue Mountains, is 2,800 feet above sea-level. The principal foot-rot-infested area is in the center of the upper valley (Sand Ridge) centering about the towns of Alicel and Imbler. In pioneer days this was rich grassland. Most of the native vegetation has vanished, but various prairie lupines, *Gaillardia aristata* Pursh, *Lithospermum ruderale* Dougl., and *Delphinium menziesii*, typical of the Festuca consociation, are still fairly common along roadsides adjacent to foot-rot-infested fields. Moreover, a dozen miles farther up the valley near Elgin, where foot rot also occurs, the low hills adjacent to foot-rot-infested wheat fields are covered with vegetation similar to the Festuca consociation of eastern Whitman County, Washington (7). *Balsamorhiza sagittata,*

Clematis hirsutissima Pursh, *Sisyrinchium douglasii, Brodiaea grandiflora* Smith, *Festuca idahoensis* and *Agropyron spicatum* are present.[6] *Paeonia brownii,* a very restricted species, is reminiscent of Tygh Ridge. *Lupinus sulphureus* Dougl. is the most abundant species of Lupinus near Elgin.

Anatone Area

This area, which is in Asotin County, Washington, was found during the summer of 1932. It occupies a long strip of territory lying on the Asotin Highlands just below the edge of the Ponderosa[7] pine (*Pinus ponderosa* Dougl.) belt. The elevation is 3,600 feet, the soil a dark, very fine sandy loam,[8] and the vegetation that of a sod-grass prairie. *Balsamorhiza sagittata* and *B. incana* Nutt., *Lupinus laxiflorus, Astragalus reventus* and *Lithospermum ruderale* occur. The flora of rocky outcrops is dominated by *Wyethia amplexicaulis* Nutt., *Delphinium menziesii,* and *Phlox viscida* E. Nels. The early spring flora contained *Tellima tenella* and *Lomatium cous.*

Spokane Valley Area

The Cercosporella foot rot occurs on a considerable acreage in the Spokane River Valley extending from Millwood, Washington, to a point beyond Post Falls, Idaho, and on Peone and Pleasant Prairies, northeast of Hillyard, Washington.[9] The soil of the Cercosporella area in the Spokane Valley is Garrison gravelly loam, containing a high percentage of small to midsize gravel. Probably because of the nature of this soil, the grass cover tends to form a bunch-grass type.[10] Characteristic plants are typical of the Festuca consociation (Fig. 2, B). Prominent plants near Liberty Lake Junction, Washington, were *Sisyrinchium douglasii, Balsamorhiza sagittata, Gaillardia aristata, Phlox longifolia* Nutt., *P. rigida* Benth., *Delphinium menziesii, Eriophyllum lanatum, Lomatium* spp., and *Lupinus sericeus* var. *flexuosus* (Lindl.) C. P. Smith. The original vegetation on the prairies north of the Spokane River was similar to that on the gravelly soil, but was more truly of the Festuca type. On the parts of these prairies where foot rot occurs, the soil is fine sandy loam of several related subtypes

[6] As in other Cercosporella areas, *Poa sandbergii* has survived the inroads of grazing animals. *Festuca idahoensis* and *Agropyron spicatum* were found in sheltered places.

[7] Foresters and lumbermen are almost universally agreed on calling *Pinus ponderosa* the Ponderosa pine rather than the older name of western yellow pine.

[8] The soil near Anatone probably represents the same general soil type as the black earth belt of Russia.

[9] Late in the summer of 1932 an area in Spokane County, west of Spangle and Plaza, also was reported. This location has not been surveyed by the writer.

[10] *Agropyron inerme* (Scribn. and Smith) Rydb. and *A. riparium* Scribn. and Smith, together with *Festuca idahoensis, Poa sandbergii* and *Koeleria cristata* (L.) Pers. produce a luxuriant growth of grass in unmolested parts along the railroad right-of-way.

TABLE 1.—*A list of the most common native plants growing in Cercosporella-infested prairie soil, Oregon and Washington. Presence indicated by positive (+) sign*

Species	Locations									Total
	Oregon					Washington				
	Seven Mile Hill	Tygh Ridge	Friend	Alicel	Elgin	High Prairie	Spokane Valley	Peone Prairie	Anatone	
Koeleria cristata	+	+	+	+	+	+	+	+	+	9
Festuca idahoensis	+	+	+	+	+	+	+	+	+	9
Agropyron inerme		+	+	+		+	+	+	+	7
Agropyron spicatum	+	+			+					3
Poa sandbergii	+	+	+	+	+	+	+	+	+	9
Brodiaea grandiflora	+	+	+	+	+	+	+	+	+	9
Fritillaria pudica	+	+	+	+	+	+	+			7
Sisyrinchium douglasii	+	+			+	+	+			5
Eriogonum spp.	+	+	+		+	+			+	6
Paeonia brownii		+			+				+	3
Delphniium menziesii	+	+	+	+	+	+	+	+	+	9
Tellima parviflora Hook.	+	+	+	+	+	+	+		+	8
Sieversia ciliata (Pursh) G. Don.	+	+				+	+		+	5
Lupinus laxiflorus	+	+	+			+			+	5
L. sericeus var. *flexuosus*							+	+		2
L. leucophyllus	+	+	+		+	+				5
Astragalus spp.	+	+				+			+	4
Lomatium geyeri	+	+				+				3
Lomatium triternatum	+	+	+	+	+	+	+	+	+	9
Frasera spp.	+	+	+			+	+			5
Phlox longifolia		+					+			2
P. viscida						+			+	2
P. speciosa Pursh	+					+	+			3
P. rigida		+					+	+		3
Hydrophyllum capitatum Dougl.	+		+		+	+			+	5
Lithospermum ruderale	+	+	+	+	+	+	+	+	+	9
Balsamorhiza deltoidea	+	+	+			+				4
B. sagittata				+	+		+	+	+	5
Eriophyllum lanatum	+	+			+	+	+	+		6
Gaillardia aristata		+	+	+			+	+		5
Senecio spp.	+	+	+		+	+	+	+	+	8

(6). All of the foot-rot areas in Spokane County and adjacent Idaho are surrounded by Ponderosa pine forests, which, in turn, merge with other conifer associations as the Rocky Mountains are approached.

The small foot-rot-infested area near Coeur d'Alêne, Idaho, occurs on logged-off land that originally was covered with fir and Ponderosa pine. This is one of the few instances where foot rot has "advanced" beyond the adjacent grassland areas.

INDICATOR PLANTS

The more prominent native plants that occur in the several foot-rot areas are listed in table 1. Six of these occur generally and may be taken as indicator plants. They are *Festuca idahoensis* (dominant), *Agropyron inerme* and *A. spicatum*, *Balsamorhiza deltoidea* and *B. sagittata*, *Delphinium menziesii*, *Lomatium triternatum* (Pursh) Coult. and Rose, and *Lithospermum ruderale*. All six occur on Seven Mile Hill, Oregon, on Tygh Ridge, and Friend, Oregon, on High Prairie, Washington, near Imbler, Oregon, and in Spokane County, Washington.

Cercosporella should not be expected to occur in all soils that once harbored these particular plants, since these plants occur over some range of climatic and soil factors. Nor does Cercosporella entirely restrict itself to the prairie areas where these plants are found. The exceptions to the occurrence of *Cercosporella herpotrichoides* in association with this particular plant consociation are so few, however, as to strongly suggest that the Cercosporella foot rot will remain a more or less localized problem.

SUMMARY

The Cercosporella foot rot occurs almost entirely in prairies that originally bore a Festuca sod-grass consociation. Indicator plants are *Festuca idahoensis* (dominant), bunch grass (*Agropyron* spp.), *Balsamorhiza* spp., *Delphinium menziesii*, *Lomatium triternatum*, and *Lithospermum ruderale*.

The writer is deeply indebted to Dr. Helen Gilkey for the checking of seed plant collections made from the foot-rot areas and to Dr. A. G. Johnson for aid in the preparation of the manuscript. Representative specimens of the vegetation of the several Cercosporella areas are deposited in the Herbarium of the Department of Botany, Oregon State College, Corvallis, Oregon.

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THE MYRIOGENOSPORA DISEASE OF GRASSES

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Clumps of living Andropogon received in July, 1931, from Bogalusa, Louisiana, had stunted and twisted shoots, this abnormal condition presumably being due to the fungus, Myriogenospora, which was abundantly present. In an attempt to propagate the fungus the diseased grasses were planted in six 6-inch pots and kept in a high temperature greenhouse (70–100° F.) until June, 1932, after which time they were kept out of doors until July, 1933. Although all the clumps survived the transplanting, only one continued to bear the fungus and the diseased condition throughout the season of 1932. In 1933, however, the infection was no longer evident and this clump showed only a healthy condition. The continuance of the infection through 2 seasons has, however, afforded an opportunity to observe a little-known but distinctive pathological effect that seems worthy of record.

Throughout the period from July, 1931, to October, 1932, almost half the clump remained normal without trace of disease, the presence of the fungus being limited to certain shoots that stood out in decided contrast because of their dwarfed and fasciated condition (Fig. 1). The diseased shoots possessed a healthy green coloration and grew vigorously; they were without any chlorotic effect except for the slightly reddish pigmentation immediately adjacent to the stromata of the fungus. On inspection the feature most notable was a characteristic adhering of leaf tips to adjacent leaves or stems that had arisen from a common axis (Fig. 2, A). This attachment seems due chiefly to the mechanical binding by the immature stroma of the fungus during the time the adjacent leaves and culms were still folded and packed together within a subtending leaf sheath. As the shoot and leaf elongated there was a separation except where held firmly together by this fungous stroma (Fig. 2, B). With many leaves and stems bound together in this manner the elongating culms also characterized by internodal dwarfing were deflected from their normal course and prevented mechanically from growing erect. Coincident with dwarfing and fasciation the inflorescences of diseased shoots were aborted or at least sterile, whereas the near-by healthy shoots bore normal florets. The mass of culms and leaves so dwarfed and distorted present an appearance strikingly similar to that recorded for the "Pokka Bong" disease of sugar-cane, which is due to a different fungus.

The record by Vizioli[1] and that by Freise[2] of *Myriogenospora aciculi-*

[1] Vizioli, Jose. Estudo preliminar sobre un novo pyrenomyceto parasita da canna. Bol. Agr. São Paulo 27: 60–69. 1926.

[2] Freise, F. W. Cane diseases and plagues in Brazil. Facts about Sugar 25: 613–614. 1930.

FIG. 1. Clump of *Andropogon scoparius* infected by *Myriogenospora paspali* and showing a fasciated and dwarfed condition. At right and rear are normal shoots which do not bear the fungus. Specimen growing in 6-inch pots in the greenhouse. ×¼. Photographed by M. L. S. Foubert.

FIG. 2. Enlargement of parts of figure 1. A. Part of fasciated plant showing the attachment of leaf tips to near-by leaf blades and culms. ×1.5. B. Folded leaf blade in region of tip adherent to black stroma of Myriogenospora along the midrib of the adaxial surface of another leaf. Note two rows of ostioles with extruding spore masses along the black stromatic surface. ×5.3. Photographed by M. L. S. Foubert.

sporae Vizioli in Brazil note a similar attachment of sugar-cane leaves by the mechanical binding of the stroma but do not describe the fasciation observable in *Andropogon scoparius*. In so far as the writer has been enabled to observe grasses in the southern United States affected by Myriogenospora, the fasciation is most severe upon Andropogon, although the effect upon certain other grasses is pronounced. Freise's (*l.c.*) account of the Myriogenospora disease of sugar-cane in Brazil indicates local severity but of minor economic consequence. Myriogenospora is not known from the eastern hemisphere, but that the genus is widespread upon Andropogon, Paspalum, Axonopus, and Panicum in tropical and subtropical America east of the Cordilleras is attested by herbarium specimens. Such records, however, convey no hint of the severity or prevalence of the disease.

It was thought that the inoculum provided by the diseased clump of Andropogon might be used to indicate how the diseased condition is engendered in nature. Hence, during the autumn of 1931 various attempts were made to reinfect artificially the 5 clumps of healthy *Andropogon scoparius* that had previously shown the disease. Attempts were made also to infect an equal number of potted clumps of the same species transplanted from near Falls Church, Virginia, as well as 5 plants of *Paspalum dilatatum* grown from seed in the greenhouse. During the same season and the succeeding winter attempts were likewise made to infect sugar-cane, using for a medium P. O. J. No. 228,[3] which is the clone recorded by Vizioli (*l.c.*) as host for his *M. aciculisporae*. These inoculations were all carried on in the greenhouse under the same high temperature conditions (70–100° C.) which prompted germination of ascospores in water. In no case was there any success in securing infection. The methods used were as follows:

1. Spraying over all of a tap-water suspension of germinating ascospores, followed, except for the sugar-cane, by being kept for 48 hours in a darkened moist chamber.

2. Hypodermic injection of germinating ascospores in tap-water suspension into leaf spindles, nodes, and internodes of the various grasses noted.

3. Placing of bits of stromata bearing germinable ascospores between the leaf sheaths and culms.

The fungus concerned is here called *Myriogenospora Paspali* Atk., although there may properly be some question as to this identification, since most records of the fungus upon Andropogon refer it to *M. Bresadoleana* P. Henn., a species also originally described on Paspalum. The 3 recorded species of the genus *M. Paspali*, *M. Bresadoleana*, and *M. aciculisporae* Vizioli are not described as essentially different. The slight differences in the recorded asci and spore dimensions for these species do not distinguish

[3] Thanks are due to Dr. E. W. Brandes and Dr. Julius Matz for the initial plants of P. O. J. No. 228.

them. Moreover, the actual dimensions of asci and spores in living material of this fungus on Andropogon might identify it with either of these 3 species. The identification of this fungus on Andropogon with Atkinson's species must, therefore, be tentative.

DISCUSSION AND CONCLUSIONS

The loss of the diseased condition coincident with the disappearance of the fungus, *Myriogenospora Paspali,* suggests a causal relationship and shows definitely an example of recovery of a grass from a partially systemic disease.

Since there is no chlorosis or early death of infected shoots, the dwarfing and fasciation, as well as sterility, of the grass accompanying the fungus are nevertheless of peculiar pathological significance.

The fasciation appears to be due chiefly to a mechanical binding by fungous tissue of adjacent leaves and culms during the growth of the shoot.

The artificial inoculations, while ineffective, suggest that with the particular strain used infection by Myriogenospora may not take place except through the seed at the time of seed setting or else at a period of susceptibility when seeds germinate. Neither of these two types of infection could, however, have taken place in Brazil with the sugar-cane clone P. O. J. No. 228, because of its Javanese origin and its apparent freedom, except in South America, from infection by the fungus. *Myriogenospora aciculisporae* Vizioli would, therefore, appear, in the absence of reports elsewhere, to behave differently from the fungus on Andropogon. The fact that in the Southern United States Myriogenospora is not known on sugar-cane, although it is not uncommon on native grasses in the same region, suggests an immunity from available strains of the fungus in the clones grown there.

MYCOLOGY AND DISEASE SURVEY
BUREAU OF PLANT INDUSTRY

NECTAR AND RAIN IN RELATION TO FIRE BLIGHT

H. Earl Thomas and P. A. Ark

(Accepted for publication August 17, 1933)

Several workers during the past 15 years (2, 3, 5) have noted that the dissemination of the fire-blight organism, *Bacillus amylovorus,* is increased during wet weather, and some have adopted the view that splashing rain is the principal agent of dissemination. While it is a demonstrable fact that rain water may carry the organism from a holdover canker to blossoms on the same tree, there is no clear evidence that a wider dissemination is due to this agency.

The nectar of fruit-tree blossoms has not been critically studied in relation to fire blight, although it has long been accepted that the organism multiplies in the nectar and frequently penetrates through the nectary into the blossom.

In earlier work with the fire-blight disease, the writers shared the seemingly common impression that the nectar of fruit-tree blossoms rarely exceeds 20 per cent in concentration of total sugar. In the spring of 1932, George H. Vansell[1] called the attention of the writers to the work of Miss Beutler (1) in Germany and to his own unpublished work (6) in California, showing that the concentration of sugar in the nectar of fruit trees may vary from 2.3 per cent to 55 per cent or even higher. It was further shown that the variation in sugar concentration stands in close (inverse) relation to the atmospheric humidity and that the volume of nectar varies widely in direct relation with humidity.

The question at once arose whether the fire blight bacteria would be capable of multiplication in these more concentrated sugar solutions. Some tests were, therefore, made to determine the maximum concentration of the sugars dextrose, levulose, and sucrose,[2] that the organism will tolerate in culture solutions. Solutions were prepared by adding the desired quantity of sugar to the following nutrient solution adjusted to pH 6.9: magnesium sulphate, 2 gm.; dipotassium phosphate, 1 gm.; asparagin 3 gm.; distilled water 1000 gm.

Ten different cultures of the blight organism from as many sources were grown in each concentration of sugar. In dextrose solutions growth was optimum at 3 per cent, feeble at 14 per cent and very slight at 24 to 28 per cent. Several cultures made no growth at 16 and 18 per cent. In solutions of levulose, the highest concentration that permitted any growth was

[1] Associate Apiculturist, Bureau of Entomology, United States Department of Agriculture.

[2] The sugars that are found in nectars.

18 per cent and the optimum was near 10 per cent. In sucrose solutions growth was optimum at 10 per cent, but all cultures made feeble growth at 50 per cent and 2 cultures made very feeble growth at 58 per cent. It was found that the organism is able to survive for several weeks even at 60 per cent of sucrose but without perceptible growth and, upon recovery, is distinctly attenuated, requiring several passages through green pear fruits to regain its original virulence. It may be pointed out that the nutrient solution used in these tests, aside from excess of sugar, is highly favorable to the growth of the organism, while nectar is held to be low in all solutes except the sugars. In preliminary tests with pure extracted honey, which contains chiefly reducing sugars, a dilution of 1 to 8 in water was necessary before appreciable growth of the organism was obtained. In general, the concentration of sucrose, which inhibits the organism, is approximately double that of dextrose and levulose, a difference that is more or less parallel with the difference in the osmotic values of these sugars. However, the 2 reducing sugars seem to possess relatively somewhat stronger bacteriostatic power than is referable to simple osmotic relations.

It is apparent from these results that the maximum total sugar concentration tolerated by the organism will depend upon the proportion of the different sugars present in the nectar. Beutler presents some figures showing the relative amounts of saccharose and invert sugar for apple, crab-apple, cherry, and plum and states that the nectar of fruit-tree blossoms in general contains these sugars in a ratio of about 1:1. The writers have found no definite record in the literature for the sugar content in the nectar of pear blossoms.

Three samples of nectar from Bartlett pear blossoms collected at different dates were analyzed through the kindness of Mr. V. W. Smart of the Division of Plant Nutrition. In 2 of these no sucrose was detected and in the third only 11 per cent of the total sugar was measured as sucrose. Single samples of quince and apple nectar yielded 29 and 34 per cent sucrose, respectively. The method of Benedict and Osterberg as adapted by Thomas and Dutcher (4) was used in these analyses. Even disregarding the limited volume of nectar secreted in a dry atmosphere, it seems unlikely that the blight organism will increase appreciably in pear nectar after the concentration of sugars reaches 20 to 30 per cent. The comprehensive tests (by refractometer) of Vansell and a considerable number of determinations by the writers, indicate that higher concentrations are by no means infrequent in the comparatively dry air of the principal pear-growing areas of California. Vansell found pear nectar so low in volume at Davis and Placerville in March and April, 1932, as to preclude satisfactory sampling.

A few observations and tests were made at Berkeley in 1932 bearing on

the amount and concentration of nectar in pear, apple, quince, and cherry blossoms. These were examined from time to time during the early part of April but at no time was nectar found in abundance. Indeed, several attempts to obtain, under natural conditions, a sufficient quantity of nectar for refractometer determinations, met with indifferent success. However, when detached blossoms of apple, quince, and cherry were placed over night in an atmosphere of high humidity, nectar was produced in considerable volume, and the total sugar concentration under these conditions ranged from 6 to 11 per cent.

During the blossoming season of 1933, nectar was found in measurable quantities in pear blossoms at Davis and Berkeley. Both were on sunny days with comparatively dry atmosphere after early morning, and in both instances the total sugar concentration changed from 8 to 10 per cent at 9:00 a. m. to 20 to 22 per cent at noon after which samples were not obtainable by pipette. Samples of nectar taken from bees working in pear blossoms between 3 and 4 o'clock of the same day (at Davis only), contained 38 to 50 per cent of total sugar. On a drier day more nearly typical of that blossom period more than 100 blossoms were required at 6:30 a. m. to yield sufficient nectar for a refractometer reading, which, in this case, was equivalent to 18 per cent sugar. The rate of change from concentrated to dilute nectar has not been followed in the field. Detached pear blossoms, collected during a dry period and placed in an atmosphere nearly saturated with moisture, yielded nectar containing 5 per cent of sugar after $4\frac{1}{2}$ hours, about 4 per cent after 7 hours, and $2\frac{1}{2}$ per cent after 48 hours. In one instance the nectar was collected from 75 pear blossoms after these had been kept for 63 hours in a moist chamber. These averaged 1 cubic centimeter of nectar for each 25 blossoms and the concentration of sugar was less than 2 per cent.

Pear nectar has not been found in the orchard in sufficient volume to be studied as a culture medium. Nectar from plum sterilized by filtration and containing 3.2 per cent sugar, permitted vigorous growth of the fire-blight organism.

Apple blossoms were brought into the laboratory and atomized with a suspension of the fire-blight organism. These were then divided into 2 lots. One of these was dried for an hour in the draught from an electric fan, and the other was placed in an atmosphere of high humidity. The nectar in the latter soon increased to a large drop in each blossom with a total sugar concentration of about 5 per cent. After 2 days the filaments in contact with the nectar were discolored and in 5 days typical symptoms of blossom blight were apparent. The blossoms kept in dry laboratory air remained free from symptoms 7 days after the time of inoculation, when they were discarded.

In 2 similar experiments with pear blossoms, similar results were obtained. In a third test approximately half of the blossoms in the laboratory air became blighted, while all of those in the more humid atmosphere developed symptoms. The fan was not used to hasten drying in the experiments with pears.

It is probably significant that the principal blossom periods of pears in the Sacramento Valley in 1932 and 1933 were accompanied by prevailing dry winds and very few blossom infections. It also is of interest that in the epiphytotic season of 1930, precipitation was below normal at Sacramento for April, May, and June but atmospheric humidity was unusually high at certain periods.

The foregoing facts seem to the writers to offer a more reasonable explanation for the observed acceleration of fire blight during periods of high relative humidity than does the assumption that rain functions directly as a principal vector.

SUMMARY

The nectar of fruit-tree blossoms grown in a dry atmosphere is found to contain sugars in concentrations considerably greater than those that will permit growth of the fire-blight organism in culture solutions. It is proposed that the increase in volume and the reduction in concentration of nectar during humid weather bears an important relation to the incidence of fire blight.

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PHYTOPATHOLOGICAL NOTES

Physiologic Specialization in Puccinia glumarum in the United States.—Since the discovery of *Puccinia glumarum* (Schmidt) Eriks. and Henn. in the United States by Dr. F. Kølpin Ravn, in 1915, physiologic specialization in this species has been of special interest. Aside from the work of Hungerford and Owens,[1] in which they state "that the rust which attacks *Hordeum murinum* may not be the variety commonly found on wheat," there has been until recently little or no evidence of more than one physiologic form of *P. glumarum* in the United States.

On July 18, 1933, in the uniform stripe-rust nursery grown in the Flathead Valley of Montana, the wheat variety Red Russian (C. I. 5409) was found to be susceptible to the form of *Puccinia glumarum* prevalent in that section. This same variety, however, always has been immune from the form commonly present at Moscow, Idaho.

At the time of the nursery reading a goodly amount of the rust was collected for further study under controlled conditions of light and temperature at Moscow. The culture was increased on Pannier barley until transferred to the various wheat-variety testers for a comparison with the Moscow form. On December 5, 1933, a number of wheat varieties used by Gassner and Straib[2] in their determination of physiologic forms of *Puccinia glumarum* in Europe, together with some American varieties of wheat and emmer, were inoculated with the Montana culture. After inoculation the plants were kept in a temperature-and-light controlled room at 52–55° F. and were given 12 hours of light, daily, supplied by a 500-watt electric light. They were exposed to the above conditions for a 30-day period and then were read for their reaction to the rust. Later, different plants of the same varieties were inoculated with the form of *P. glumarum* common to Moscow, Idaho, and were exposed to the above-mentioned environmental conditions. The results of these two tests are given in detail in the following table.

From the above table it is apparent that the varieties Red Russian and Chinese 166 differ decidedly from the other varieties in the reaction to the two collections of *Puccinia glumarum*, both being resistant to the Moscow collection and susceptible to the one from Montana.

Because of these differences and because the two collections were tested under identical environmental conditions, the writer believes there is suffi-

[1] Hungerford, Charles W., and C. E. Owens. Specialized varieties of *Puccinia glumarum*, and hosts for variety *tritici*. Jour. Agr. Res. 25: 363–402. 1923.

[2] Gassner, G., und W. Straib. Die Bestimmung der Biologischen Rassen der Weizengelbrostes (*Puccinnia glumarum* f. sp. *tritici* (Schmidt) Erikss. u. Henn.). Arb. Biol. Reichsanst. Land- u. Forstw. 20: 141–163. 1932.

TABLE 1.—*The reaction of a number of wheat varieties to two stripe-rust collections*

Variety	Moscow, Idaho, collection			Flathead Valley, Mont., collection		
	No. of plants inoculated	No. plants showing infection	Response[a]	No. of plants inoculated	No. plants showing infection	Response
Webster (C. I. 3780)	10	6	R	9	7	R
Red Russian (C. I. 5409)	9	0	A I	11	5	S
Triplet (C. I. 5408)	10	2	S	12	6	S
B. Winter Emmer (C. I. 2337)	11	10	S ++	12	6	S ++
Heines Kolben	14	10	S	12	7	R –
Vilmorin's Blé rouge d'Écosse	11	0	A I	11	0	A I
Rouge Prolifique barbu	12	0	A I	9	0	A I
Chinese 166	13	1	R ++	13	9	S ++
Holzapfels Früh	11	0	A I	11	0	A I
Spaldings Prolific	10	0	A I	12	0	A I
Michigan Amber 29–1–1–1	14	1	S	12	7	S ++
Strubes Dickkopf	10	0	A I	11	0	A I
Vilmorin 23	13	0	A I	9	0	A I
Carstens Dickkopf V	9	0	A I	11	0	A I

[a] A I = Apparently immune; R ++ = extremely resistant; R = moderately resistant; R – = fairly resistant; S = moderately susceptible; S ++ = completely susceptible.

cient evidence to warrant the conclusion that they represent two distinct physiologic forms, the Montana form being the one evidenced for the first time in the United States.

A more detailed report of these findings will follow.—WAYNE M. BEVER. Cooperative investigation between the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Crown Gall of Hops.[1]—Crown gall of hops has undoubtedly been present in Pacific Coast yards for many years. The association of *Phytomonas tumefaciens* (Smith and Town.) Committee S.A.B., with the disease has been definitely recognized since 1907. Smith, Brown, and Townsend made exhaustive preliminary studies of the organism isolated from galls on a number of plants, including hops. By means of artificial inoculations with the organism obtained from hop galls they reproduced the disease on almond, daisy, grape, hops, sugar-beet, and tomato plants. Their attempts to infect cotton, olive, and peonia were unsuccessful. The crown-gall organism from daisy galls was successfully transmitted to English Cluster and Custis Late varieties of hops. The organism from peach galls likewise was transferred to hops. These authors state, "The reports of hop growers on the Pacific Coast indicate that this disease may do considerable damage, particularly as the galls often reach a diameter of one's double fist. Some believe that an attack of two years' duration is sufficient to kill a plant. According to Dr. W. W. Stockberger of this Bureau, the disease occurs on hops not only in Washington State and Oregon, but also in the Sacramento valley in California. There I have seen acres of hops in which scarcely a hill could be found which did not show these tumors, some of them being larger than my first."[2]

What appears to be the first official report to the Plant Disease Survey of the United States Department of Agriculture of the presence of the crown gall of hops on the Pacific Coast was made on September 1, 1929, when a 35-acre Polk county, Oregon, yard of the Late Clusters variety was found in which some 10 per cent of the plants were infected.[3] Subsequent meager reports of the incidence of the disease on the Pacific Coast disclose the fact that both the Early Clusters and Late Clusters varieties have been found infected in Benton, Linn, and Polk counties, Oregon, each year since 1929 to date. The so-called Bavarian hops also have been reported infected.

[1] Published as technical paper No. 221, with the approval of the Director of the Oregon Agricultural Experiment Station.

[2] Smith, E. F., Nellie A. Brown, and C. O. Townsend. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr. Bur. Pl. Indus. Bul. 213. 1911.

[3] Report made by S. M. Zeller, Oregon Experiment Station, Corvallis.

A specimen of the disease on Late Clusters was taken in Sonoma county, California, in 1933.

Early in March, 1932, sexless plants of the Late Clusters variety were being rogued from a yard consisting of several hundred acres located in Polk county, Oregon. The roots of 400 of these rogued plants were examined, and 6.25 per cent were found to be infected with crown gall. Many of the galls were very extensive and involved entire crowns.

The vegetative characteristics of the host plant, which develops a perennial rootstock, is such that, in older plantings particularly, a high percentage of the plants might be infected without the fact becoming evident, or any appreciable damage being done, unless entire crowns become diseased and the development of all normal shoots from such diseased crowns were thereby prevented.

In hop nurseries, where underground stem cuttings are rooted and allowed to remain a year or more before being planted out in the main yard, crown gall is occasionally encountered in quantities sufficient to necessitate the destruction of a considerable amount of planting stock.

Otherwise, the disease, while doubtless quite general, cannot be considered of major economic importance.

The customary cultural practices to which hops are subjected afford ample opportunity for the spread of the disease. It undoubtedly is spread from yard to yard by planting infected or contaminated cuttings. Several specific cases are on record that would seem to confirm this statement. It is possible also that nematodes may be associated with the spread of the disease.

The writer recognizes 3 distinct types of gall: one of which involves the entire crown, and is comparatively uncommon. A second type of gall usually involves buds on the underground stems, and produces "hairy-root." The third and most common type of gall affects isolated portions of the underground plant parts or a portion of the crown at or near the ground level. In the field, the hairy-root type of gall disintegrates very readily, which also is true of the other 2 types when these growths occur on the crown near the soil surface and are subjected to severe winter weather.

What is presumed to be the causative organism has been isolated from both the second and third types of galls on Early Clusters, and Late Clusters varieties of hops. When the organism recovered from the last type of gall mentioned was used for artificial inoculations in the greenhouse, a similar type of gall was produced within 30 days on the crowns of both Fuggles and Late Clusters varieties.

It is of interest to note that A. J. Riker, to whom specimens of the second and third types of galls were sent, reported, after several trials, the inability of his associates to isolate a causative organism and suggested that

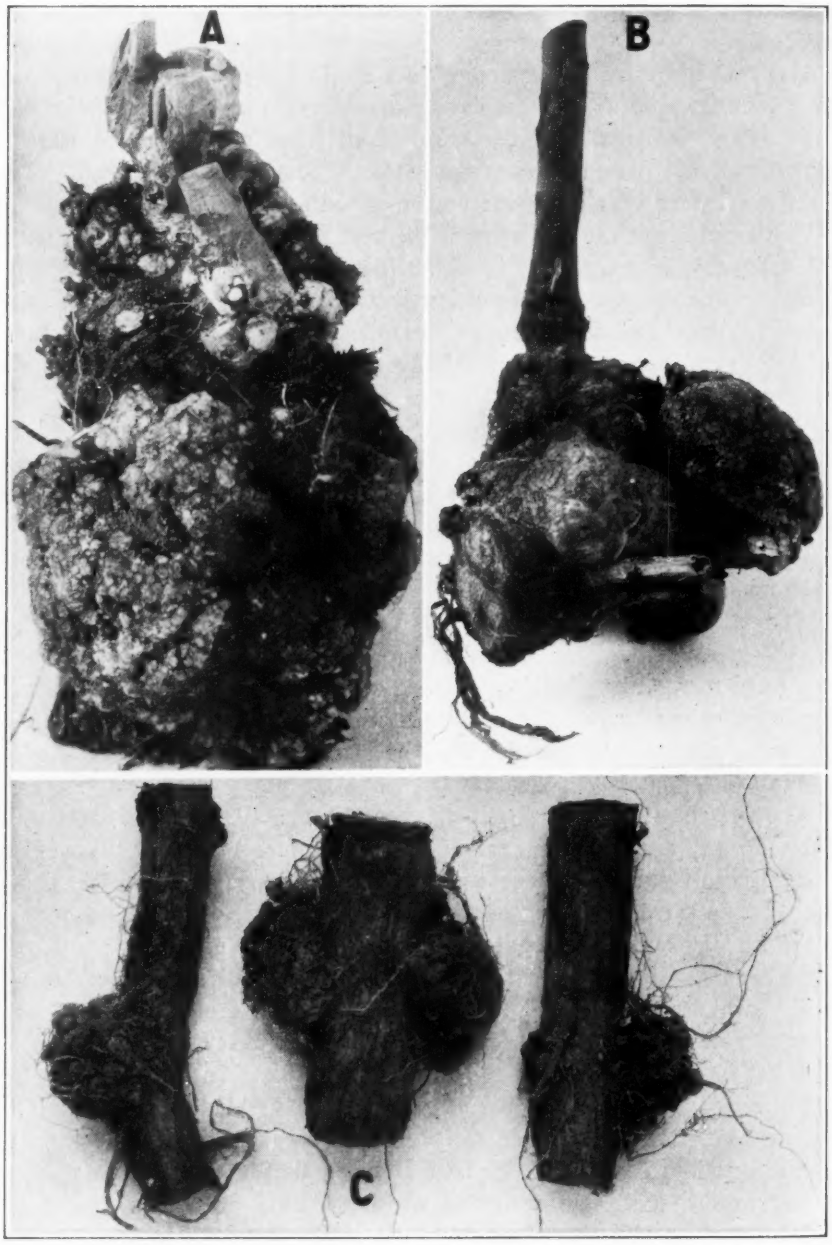

FIG. 1. Crown gall of hops. A. Exceptionally large gall on Early Clusters. B. Typical gall on Late Clusters. C. Hairy-root type of gall on Early Clusters.

the galls might be overgrowths brought about by some unfavorable physiological conditions rather than of bacterial origin. Later isolations made under Dr. Riker's supervision from the third type of gall resulted in the successful recovery of an organism with which tomato plants were artificially inoculated and similar galls produced.

Further and more detailed investigational work with this strain of the crown-gall organism from hops is being initiated.—G. R. HOERNER,[4] Oregon Experiment Station, Corvallis, Oregon.

Methods for Determining Sex Differences in Sphacelotheca Sorghi.—In genetic studies on *Sphacelotheca sorghi* (Link) Clinton, it is important to know the sexual capabilities of primary monosporidial lines, since paired lines of opposite sex are prerequisite to normal infection and production of chlamydospores in the host plant (5).

Heretofore, two methods have been used for determining sex in *Sphacelotheca sorghi:* 1. Rodenhiser (5) inoculated young sorghum plants hypodermically, in the greenhouse, with paired and nonpaired monosporidial lines and found that certain combinations of lines produced chlamydospores and others did not. This is a reliable test but requires considerable labor and time in waiting for results; 2. Rodenhiser obtained preliminary results indicating that when young sorghum plants were inoculated hypodermically the chlorosis of vegetative parts was indicative of the sexual compatability of the lines used in inoculating. The writer has confirmed these results through extensive experiments. This second method has the advantage of making it possible to get results in a shorter time than by the first method.

In addition to the two methods already mentioned the writer has utilized sporidial fusions as a sex index in *Sphacelotheca sorghi,* as various workers have done with other smut fungi (1, 2, 3, 4, 6). Isenbeck, in unpublished data obtained at the University of Minnesota, stated that he observed rather scanty fusion between sporidia of *S. sorghi* in mixed cultures of monosporidial lines grown on nutrient agar. The writer observed numerous sporidial fusions in cultures of single chlamydospores and certain paired monosporidial lines on potato-dextrose and malt agar on cover slips over van Tieghem cells. The detection of fused sporidia is complicated by the presence of numerous non-fused cells and also by the types of fusion involved. Different stages of fusion were observed. Cultures of young, rapidly budding sporidia seem to be prerequisite to successful fusion, as very few or none were observed in mixtures of old stock cultures. This method is reliable and time-saving, but requires considerable experience.

A still simpler method is that used by Bauch on *Ustilago violacea*

[4] Agent, Division of Drug and Related Plants, United States Department of Agriculture.

(Pers.), *U. scorzonerae* (Alb. et Schw.), and *U. zeae* (Beckm.) Unger (1, 2, and 3). The method follows: Four primary sporidial lines are isolated from a single chlamydospore. One line is grown in combination with the other 3 on slightly alkaline malt agar in a Petri dish. After incubation at the proper temperature, sex differences are indicated by the development of a fluffy white mycelium, the so-called *Suchfäden*. Simulating Bauch's technic, the writer obtained excellent results with *Sphacelotheca sorghi* (Fig. 1). Also, it was found that slightly alkaline potato-dextrose agar was

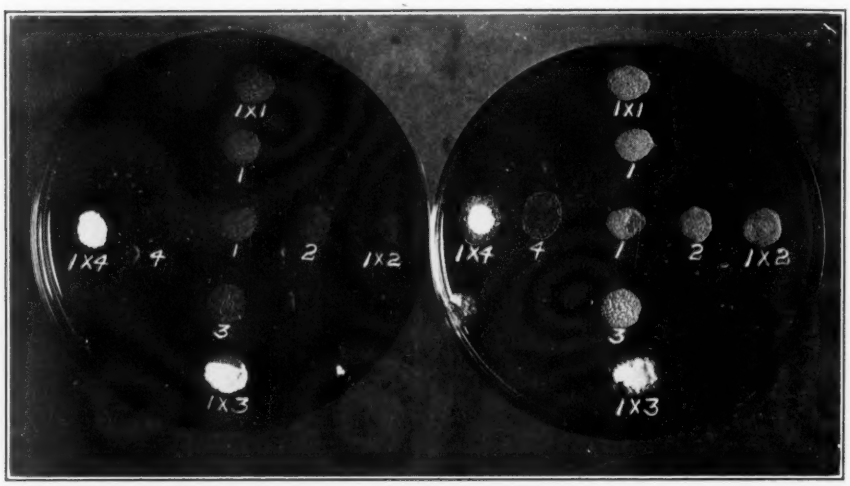

FIG. 1. Duplicate agar plates showing the results of the Bauch test on four primary monosporidial lines from a single promycelium of *Sphacelotheca sorghi*. Line 1 is the tester; material from the center colony is smeared on the colonies of lines 1, 2, 3, and 4 in the outer circle, while the colonies of the same lines in the inner circle are left as checks. It is evident that line 1 is of different sex than lines 3 and 4, while 2 is like 1. These results were confirmed by the sporidial-fusion method and by inoculating plants.

satisfactory, although the results were not so sharp as on Bauch's medium. In mixtures of those lines that reacted positively in the Bauch test there were fused sporidia as well as *Suchfäden*, while there were none in the checks. On the leaves of sorghum plants inoculated with lines that reacted positively on the Bauch medium, there were chlorosis and flecking, and, later, as the plants approached maturity, chlamydospores were produced. Lines with negative Bauch reaction also gave negative results in inoculated plants.

Holton (4) obtained inconsistent results with the Bauch test as applied to *Ustilago avenae* and *U. levis*. It is possible that he deviated too much from the procedure recommended by Bauch. In the writer's experience the method so far has been entirely reliable for *Sphacelotheca sorghi*.—LEON J. TYLER, University Farm, St. Paul, Minn.

1. BAUCH, R. Kopulationsbedingungen und Sekundäre Geschlechtsmerkmale bei *Ustilago violacea*. Biol. Centralbl. **42**: 9–38. 1922.
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A Procedure for Inducing the Production of the Sporangial and Swarm Stages in Certain Species of Phytophthora.—Investigations with *Phytophthora* spp. have been greatly facilitated by the development of a method for inducing quickly and at a desired time the production of an abundance of zoospores. Mycelium from nutrient-agar slant cultures is transferred to weak prune-juice broth (60 gm. prune pulp in 1,000 cc. tap water) and the fungus incubated at, or 2° to 4° C. below, its optimum temperature for a period of 10 days to 2 weeks, when considerable growth will have been made. The mycelium is next transferred to a Petri dish having an amount of sterile tap water (about 8 cc. in a 95 mm. Petri dish), which will submerge approximately half of the mycelial transfer, permitting some hyphae to protrude above the water level. Zoosporangia form in large numbers; in the case of *Phytophthora citrophthora* they begin to make their appearance in 12 to 15 hours. Incubation in light appears to favor sporangium formation in this species.

Division of sporangial protoplasts into zoospores and emptying of sporangia are then induced by sudden exposure to 2 temperatures. The Petri dish is floated for 3 to 5 minutes in water having a temperature of 28° to 30° C. It is then transferred to water at 15° to 18° C. in which it is allowed to remain for an hour. Zoospores usually begin to emerge 15 to 20 minutes after transfer to the cooler water and are in greatest number after approximately 1 hour. Zoospores thus produced and kept at a temperature of 18° to 22° C. will retain their motility for 45 to 90 minutes. The method has been very useful in our laboratory for experiments in the prevention of infection of fruits by various disinfectants and fungicides and for testing and observing directly the effect of such substances on the motility and germination of the zoospores.

The idea of utilizing a sudden drop in temperature to induce swarming was suggested to us by our experiences with *Phytophthora* spp. in the laboratory and by the observations of H. J. Toxopeus reported by correspon-

dence from Poenten, Java, where he noted that the gummosis organism produced an abundance of zoospores during the monsoon rains that suddenly lowered the temperature.

The foregoing procedure has been successful with *Phytophthora citrophthora, P. parasitica* (*terrestris*) and *P. cactorum*. A discussion of the various factors involved in the method will be reported later. H. S. FAWCETT and L. J. KLOTZ, Citrus Experiment Station, Riverside, California.

A Correction: The Editor wishes to call attention to the fact that through an error in printing the contribution by L. O. Kunkel, entitled "Studies on acquired immunity with tobacco and aucuba mosaics" (Phytopath. **24**: 437–466. 1934), figures 2 and 7 occupy interchanged positions and each is accompanied by the legend of the other.

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